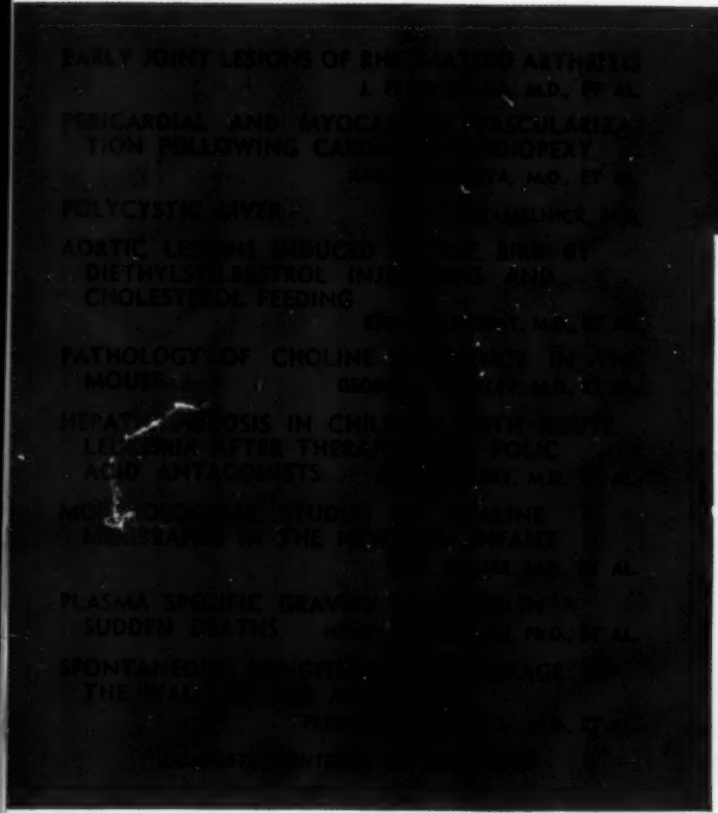
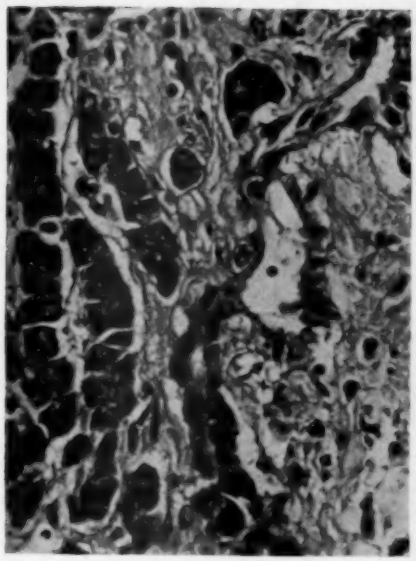
A.M.A. Archives of PATHOLOGY





AMERICAN MEDICAL ASSOCIATION Publication

distinct

cytologic diagnosis in genital and extragenital cancer

PAPANICOLAOU STAINS

EA 50, 100 cc. and 450 cc. bottles
EA 65, 100 cc. bottles
OG 6, 100 cc. and 450 cc. bottles
Harris Hematoxylin (Ortho modification) 100 cc. and 473 cc. bottles
Ortho® Dual-Purpose Cannula
Aspirator bulb (for use with Cannula)

Complete information on request.

Ortho Pharmaceutical Corporation
Raritan, N. J.

Ortho

TABLE OF CONTENTS

VOLUME 59 FEBRUARY 1955 NUMBER 2

ORIGINAL ARTICLES

REGULAR DEPARTMENTS

A. M. A.
ARCHIVES OF PATHOLOGY

VOLUME 59 FEBRUARY 1955 NUMBER 2

Published Monthly by

AMERICAN MEDICAL ASSOCIATION

535 NORTH DEARBORN STREET • CHICAGO 10, ILLINOIS

Entered as Second Class Matter Jan. 20, 1926, at the Postoffice at Chicago, Under the Act of March 3, 1879. Annual Subscription, $8.00

there's a better way to get a "normal"

Diagnostic Plasma Warner-Chilcott is a new lyophilized normal plasma that saves laboratory workers from the collector of "normal" blood. This economical preparation also brings greater confidence to technicians responsible for accurate prothrombin time determinations. It's a dependable standard, and as unvarying as is physically possible. Keep a supply on hand, especially for night or emergency requirements.

Diagnostic Plasma

WARNER-CHILCOTT

Available at leading laboratory supply houses. Write for name of your local supplier.

Supplied in boxes of ten 0.5 cc. vials, $9.00.

WARNER-CHILCOTT

Instructions to Contributors

Communications regarding editorial management, subscriptions, reprints, etc., should be addressed to Specialty Journals, American Medical Association, 535 North Dearborn Street, Chicago 10.

Articles, book reviews, and other materials for publication should be addressed to the Chief Editor of the Specialty Journal concerned. Articles are accepted for publication on condition that they are contributed solely to that journal.

An original typescript and the first carbon of an article should be provided; it must be double or triple spaced on one side of a standard size page, with at least a 1-inch margin at each edge. An article in English by a foreign author should be accompanied by a draft in the author's mother tongue. Improvised abbreviations should be avoided.

The main title of an article may not contain more than ninety characters and spaces; a subtitle may be of any length. The subtitle should be sufficiently detached from the main title that a punctuation mark as strong as a colon can be used in the Table of Contents to separate it from the main title; it must not begin with "with."

The author's name should be accompanied by the highest academic or medical degree which he holds. If academic connections are given for one author of an article, such connections must be given for all other authors of the article who have such connections.

Typographic considerations necessitate that the first paragraph of an article contain not fewer than thirty words. A Case Report must be preceded by an introductory paragraph containing at least thirty words before the details of the case are given.

Material quoted from another publication must be quoted exactly if the original is in English—spelling, capitalization, punctuation, etc., unchanged. Material taken largely from another publication must be credited to the original author, whether quoted exactly or merely abstracted. If such material is quoted indirectly, the author must be careful to leave no complete sentence of the original unaltered. Use of uncredited quotations will be sufficient cause for rejection of an article.

Each article should be accompanied by a summary in the present tense, presenting the main points of the article, but in somewhat more generalized terms than in the article itself.

If it is necessary to publish a recognizable photograph of a person, the photograph must be accompanied by written permission of the subject himself if an adult, or of the parents or guardian if a child, to publish his photograph. An illustration which has been published in another publication must be accompanied by written permission from the author and the original publisher to reproduce it in an A. M. A. Specialty Journal.

The maximum illustration allowance is ten illustrations within a total of 100 sq. inches or $100, whichever is of greater advantage to the author. When no restrictions are placed by the author upon cropping, reducing, and grouping, the publisher is often able to use twenty or more illustrations in one article with the $100 allowance. Submit sharp prints of the following sizes, 2½″ wide × 2½″ or 3½″ high, 3½″ wide × 2½″ high, and 5½″ or 7″ wide × 3½″ high, to minimize reduction and/or cropping. Oversized originals should be photographed and a print submitted. Large photomicrograph prints will be reduced in scale unless portions to be cropped are indicated by the author.

Any cut-off marks should be made on the margins or mountings rather than on the illustration itself. Charts and drawings should be in black ink on hard, white paper. Lettering must be large enough to permit necessary reduction. Glossy prints of x-rays are requested. Paper clips should not be used on prints, since their mark shows in reproduction, as does writing on the back of prints with hard lead pencil or stiff pen. Labels should be prepared and pasted to the back of each illustration showing its number, the author's name, an abbreviated title of the article, and top plainly indicated. Charts and illustrations must have descriptive legends, grouped on a separate sheet. Tables must have captions. IT IS PREFERRED THAT ILLUSTRATIONS BE UNMOUNTED.

References to the literature should be numbered in the order in which they are referred to in the text or listed in alphabetical order without numbers. A chronological arrangement, with all entries for a given year alphabetized according to the surname of the first author, may be used if preferred. References should be typed on a special page at end of manuscript. They should conform to the style of the Quarterly Cumulative Index Medicus, and must include, in the order given, name of author, title of article (with subtitle), name of periodical, with volume, page, month—day of month if weekly or biweekly—and year. Names of periodicals should be given in full or abbreviated exactly as in the Quarterly Cumulative Index Medicus. Reference to books must contain, in the order given, name of author, title of book, city of publication, name of publisher, and year of publication. Titles of foreign articles, if originally in a generally known Romance or Germanic tongue, must either all be in English translation or all be in the original language. Titles in other languages must be translated. The author must assume responsibility for the accuracy of foreign titles.

AMERICAN MEDICAL ASSOCIATION

535 North Dearborn Street **Chicago 10**

Paragon Tray Drawer Cabinet

Compact *Low Cost*

U. S. Pat. No. 2,202,047
C101—Tray Drawer Cabinet for 3 x 1 Micro Slides
Capacity 4500—18¾ x 15¾ x 4¾

FOR FILING
MICROSCOPIC SLIDES 3 x 1"
KODACHROME TRANSPARENCIES
2 x 2" SLIDES
LANTERN SLIDES
(up to 3¼ x 4¼)
PETROGRAPHIC SLIDES

When you purchase a
PARAGON TRAY DRAWER CABINET YOU PURCHASE FILING SPACE ONLY NO WASTE SPACE-EVERY INCH USED

All Paragon Tray Drawer Cabinets are manufactured in standard sizes so that any number of sections may be interlocked to form one cabinet to accommodate any number of varied slides. The dimensions of the different cabinets are the same as to length and width, varying only in height. The cabinet formed by interlocking may be 18¾ x 15¾; 18¾ x 11 or 18¾ x 5 or it may be a pyramid with the sections varying in width.

C221—Capacity 1500 Slides—18¾ x 11 x 3¾
For Filing KODACHROME TRANSPARENCIES and 2 x 2" SLIDES

SPECIFICATIONS: All Paragon Tray Drawer Cabinets are made of reinforced steel construction, olive green finish. Interlocking device enables several units to be joined into one. Each sectional unit contains removable drawers with hand grip in front and rear. Interlocking steel base obtainable whenever required. **Constructed according to rigid specifications—not merely adapted.**

Address your orders and inquiries to Dept. P.
Manufactured Exclusively by

PARAGON C. & C. CO., Inc. • 2540 Belmont Ave., New York 58, N. Y.

All of the following articles have appeared in TODAY'S HEALTH and are now available in pamphlet form.

ALCOHOLISM

an important problem in today's living

ALCOHOLICS ANONYMOUS

Written from the standpoint of a member, the basic treatment procedures are described and the psychological problems confronting the alcoholic are discussed.
4 pages, 10 cents

ALCOHOL AND CIRRHOSIS OF THE LIVER

Relationship between alcohol, diet and cirrhosis. Increasing stress on nutritional differences.
by Russell S. Boles
4 pages, 10 cents

HOW TO HELP A PROBLEM DRINKER

Understanding the alcoholic's capabilities, the necessity of help, causes of his condition.
by Edward A. Strecker and Francis T. Chambers, Jr.
4 pages, 10 cents

THE TREATMENT OF ALCOHOLISM

Tracing the steps from convincing the alcoholic that he is sick through treatment and cure.
by Lewis Inman Sharp
4 pages, 10 cents

CONDITIONED REFLEX TREATMENT OF CHRONIC ALCOHOLISM

Its place among methods of treatment today, its development and correlation with personality factors.
by Walter L. Voegtlin
4 pages, 10 cents

INSTITUTIONAL FACILITIES FOR THE TREATMENT OF ALCOHOLISM

Comparative differences, in drinking, with the last century, new establishments and methods of treatment, lack of trained personnel.
by E. H. L. Corwin
4 pages, 10 cents

THE ABOVE SIX PAMPHLETS ARE ALSO AVAILABLE IN BOOKLET FORM FOR ONLY 50 CENTS

HOW TO MEASURE DRUNKENNESS

A partial transcript of an actual courtroom case.
H. A. Heise
8 pages, 10 cents

ADDRESS REQUESTS TO:

BUREAU OF HEALTH EDUCATION
AMERICAN MEDICAL ASSOCIATION
535 NORTH DEARBORN STREET
CHICAGO 10, ILLINOIS

A New International Centrifuge

FOR THE Modern Laboratory

The new INTERNATIONAL MODEL U is more than just a Centrifuge in a cabinet. International's experienced engineering has produced a Centrifuge with years-ahead features and unmatched performance.

You will want to know more about the new aerodynamic guard bowl ventilating system—the new stainless steel interior — the stainless steel auxiliary cover that saves valuable bench space — the electric tachometer — the electric time clock — the unique air-filtering system—the spring-loaded hinge — the new vibration dampening system which results in smooth operation without the "shaker" action of over-flexible mountings — the versatility of 84 interchangeable accessory combinations.

Let us tell you more about this completely new Centrifuge, handsomely designed to fit in with your wall counters and cabinets. Write for Bulletin J explaining all these features in detail.

INTERNATIONAL EQUIPMENT COMPANY

1284 SOLDIERS FIELD ROAD, BOSTON 35, MASS.

EARLY JOINT LESIONS OF RHEUMATOID ARTHRITIS

Report of Eight Cases, with Knee Biopsies of Lesions of Less Than One Year's Duration

J. PETER KULKA, M.D.
DOUGLAS BOCKING, M.D.
MARIAN W. ROPES, M.D.
and
WALTER BAUER, M.D., Boston

THE PURPOSE of the present investigation was to study the anatomic changes in the earliest available joint lesions of rheumatoid arthritis in order to gain a better understanding of the sequence of events in the disease process. Such data are difficult to obtain, since the onset of the disease tends to be insidious and rarely gives occasion for biopsy or autopsy. Moreover, if an anatomic examination is permitted, prolonged observation of the patient is usually necessary for a reasonably certain diagnosis.

The classic anatomic studies have been concerned almost entirely with lesions from patients who had reached the slowly progressive, permanently deforming stage.* At this stage the arthritis typically has the following microscopic features: (1) proliferation of synoviocytes and subintimal fixed cells, with villous synovial hypertrophy; (2) massive subintimal lymphocytic and, at times, plasma cell infiltration, with focal juxtavascular collections, and large lymphocytic nodules having a predilection for the bulbous ends of hypertrophied villi; (3) cartilage replacement by ingrowth of connective tissue pannus, and (4) chronic inflammatory cell infiltration and proliferation of subchondral connective tissue, with erosion and penetration of the subchondral bony plate. This histologic picture tends to be diffuse throughout the joint and seems to have little in common with the focal, more specific pathologic process which characterizes the rheumatoid nodule.

Only a few joint lesions of rheumatoid arthritis which were examined microscopically within a year after their clinical onset or while activity of the disease was maximal have previously been reported. Perhaps the earliest description of such lesion is that of Allison and Ghormley in their Case 1 of "proliferative arthritis." [2] However, this case was somewhat atypical, being associated with nongonorrheal urethral discharge, and the possibility of Reiter's syndrome was not excluded. Klinge [3] studied the joints from a case of Still's disease of four months' duration and various cases of "active" chronic polyarthritis, with or without carditis. He called attention to the occurrence of fibrin deposits, "fibrinoid degeneration,† and necrosis in both the synovialis and the articular cartilage and emphasized the basic similarity of such changes to those in rheumatoid and rheumatic nodules. Fisher,[4] in the course of arthrotomies for lavage therapy, examined joints during active stages of rheumatoid arthritis. In a case of nine months' duration, he stressed the "almost constant" presence of superficial and deeper-lying areas of "fibrinoid degeneration," apparent grossly as "granular areas" on the synovial surface. He also referred to "giant cells of the Aschoff type" and likened the entire microscopic picture to that of the synovitis of rheumatic

This is Publication No. 177 of the Robert W. Lovett Memorial Foundation for the Study of Crippling Diseases.

The expenses of this investigation have been defrayed in part by a grant from the Commonwealth Fund.

From the Medical Clinic of the Massachusetts General Hospital; the Departments of Medicine and Pathology, Harvard Medical School, and the Massachusetts Department of Public Health.

* References 1 and 2.

† The terms "fibrinoid degeneration" and "fibrinoid" have had varied usage in the past. We prefer the purely descriptive expression "fibrin-like material."

fever. Steinberg [5] reported a synovial biopsy from a case of monarticular rheumatoid arthritis of one month's duration but did not state whether the diagnosis was confirmed by follow-up. The lesion showed focal collections of lymphocytes and an increase in fibroblastic activity and in the number of capillaries. In a case with typical rheumatoid arthritis of more than two years' duration, the synovitis was characterized by "abundant neutrophiles," as well as round cells. Pique and Schajowicz,[6] reporting biopsies in six "subacute" cases, including three of less than one year's duration, described the synovial membrane as grossly thickened and hyperemic with adherent masses of readily detachable "fibrinoid." Microscopically, "fibrinoid" was present in all their early cases, and they considered it of diagnostic significance. Collins [7] published a high-power photomicrograph of a small lymphocytic focus from a lesion of four months' duration. Parker and Keefer [8] reported the case of a patient who committed suicide at the height of the disease, two years after the onset. They observed interstitial and superficial fibrin, focal necrosis of the synovialis, cartilage, and subchondral fat, perivascular hemosiderin deposits, and giant cells. Probably the earliest human arthritic lesion of any type ever examined was one of 20 to 24 hours' clinical duration described by Hench [9] in a patient with palindromic rheumatism, which is frequently a manifestation of rheumatoid arthritis.[10] "Cheesy bits of coagulated material" were noted grossly in the joint cavity, and a superficial fibrinopurulent exudate, as well as neutrophile infiltration of the synovialis, was seen on microscopic examination.

Thus, most previous reports of both early and highly active rheumatoid joint lesions differ from the classic descriptions, based on long-standing lesions, in their emphasis on subacute exudative or necrobiotic changes.

Our own study is based on eight cases of rheumatoid arthritis with knee biopsies seven days to nine months after the onset of clinical involvement of the respective joints. The actual onset of the tissue changes may, of course, have preceded the appearance of symptoms or signs by an unknown period of time.‡ The diagnosis was based in every instance on the presence of chronic symmetrical polyarthritis and consistent laboratory findings. The cases varied considerably both clinically and pathologically; the specimens removed at biopsy were of necessity small, and the inflammatory process varied widely within the same joint. For these reasons, each of the lesions to be presented must be considered individually in relation to the clinical findings. The cases are arranged in order of clinical duration of the lesions at the time of biopsy.

REPORT OF CASES

CASE 1.—*Synovitis of left knee, seven days' "duration."*

E. L., a 71-year-old draftsman, had been in good health until two years before entry, when he had a seven-week episode of stiffness and pain in his spine. Thereafter, he had fleeting arthralgia in the metacarpophalangeal joints. Three weeks before entry, he developed pain in the right shoulder and both feet. He also had a weight loss of 8 lb. (3.6 kg.). The admission temperature was 100.4 F. There was some limitation of motion of the right shoulder and right knee. The tarsal and metatarsophalangeal joints were swollen, warm, and tender. The hemoglobin was 13 gm. The white cell count was 8,400, with a normal differential. The sedimentation rate (Rourke-Ernstene [11]) was 1.68 mm. per minute (upper limit of normal, 0.35 mm.). A Hinton test was negative. X-ray films of the knees showed mild degenerative changes and mild decalcification, more marked on the right. The pelvic bones showed changes characteristic of Paget's disease. The feet improved under conservative treatment, but the temperature and sedimentation rate remained elevated. Thirty-three days after admission slight stiffness was first noted in the left knee, which also lacked 25 degrees of full flexion, and five days later, an effusion appeared. Seven days after the onset of symptoms in the left knee a biopsy was performed. One month later the arthritis began to subside and all joint motion slowly returned to normal. Five months later, only slight soft tissue thickening of the left knee remained.

BIOPSY FINDINGS

Left Knee (#H-1879).—An anteromedial arthrotomy was performed, without the use of a tourniquet. The joint capsule was bulged out by an

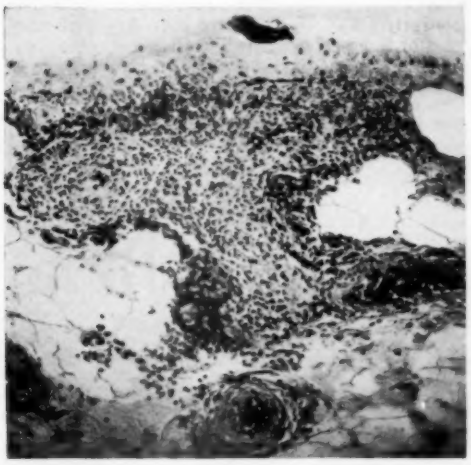
‡ To denote this uncertainty, the word "duration" is placed in quotes when referring to a lesion.

Fig. 1 (Case 1; H-1879).—Slight synoviocytic proliferation and segregated collagenous bundle at base of minute synovial villus. Note the absence of significant inflammatory cell infiltration. Hematoxylin and eosin; reduced ⅕ from mag. × 350.

estimated 30 cc. of turbid, pale yellow fluid (Table 1). The synovialis seemed smooth and slightly hyperemic. The exposed articular cartilage appeared normal.

Small pieces of parietal synovialis from the infrapatellar pouch and of visceral synovialis from the margin of the medial femoral condyle were fixed in 10% neutral formalin and Helly's fluid. Serial sections were stained with hematoxylin and eosin, Mallory's phosphotungstic acid hematoxylin, Masson's trichrome stain, toluidine blue, and Foot's reticulum stain. Frozen sections were studied for lipids and birefringence.

Fig. 2 (Case 1; H-1879).—Miliary granuloma on surface of visceral synovialis. The core of the lesion is formed by branches of the venule at the lower right, most of which is cut tangentially in this particular section. Note "button" of fibrin-like material, disruption of collagenous stroma, and patchy accumulation of ground substance, which displaces some fat cells in lower portion of field. Phosphotungstic acid hematoxylin; reduced ⅕ from mag. × 150.



The sections showed a slight, diffuse, proliferative reaction, confined almost entirely to the synovial intima (Fig. 1), and a focal, nonsuppurative,§ and granulomatous inflammation, which tended to be centered about certain subintimal venules and capillaries (Figs. 2 through 5).

The intima had a pseudostratified appearance, with a basal zone, containing two to five "layers" of nuclei, and a superficial eosinophilic granular zone of variable thickness, which appeared to consist of the elongated cytoplasmic processes of the synoviocytes. There was no appreciable amount of intracellular or extracellular Sudanophilic or birefringent material. The basal synoviocytes were rounded and showed occasional

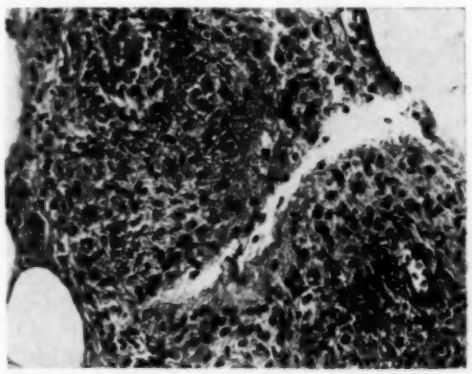


Fig. 3 (Case 1; H-1879).—Inflammation centered about blood vessels near synovial fissure or crypt. The arrow points to a capillary which is almost occluded by endothelial hypertrophy and surrounded by a delicate fibrin network, as well as by emigrating polymorphic mononuclear cells. The synovial intima adjacent to this capillary is distorted. Hematoxylin and eosin; reduced ½ from mag. × 390.

§ This term denotes that the cellular exudate is predominantly of the lymphoid series; "granulomatous" designates predominance of the reticuloendothelial series. Such reactions could hardly be referred to as "chronic" when they occur in lesions of days' or weeks' duration.

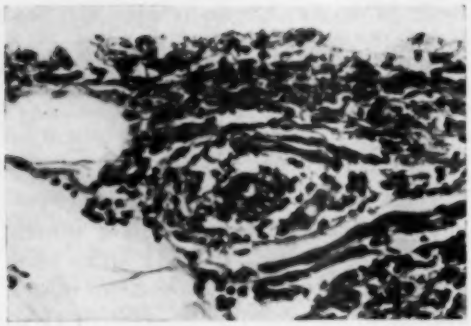


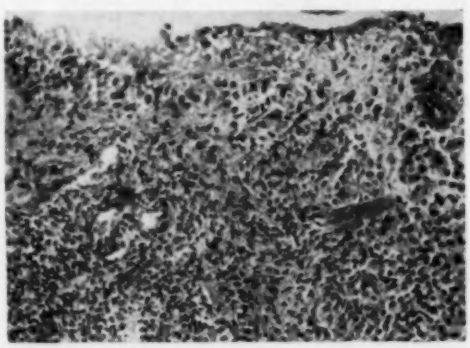
Fig. 4 (Case 1; H-1879).—Venulitis with partial luminal obstruction by small mononuclear leucocytes. Note the focal infiltration of the overlying synovialis by similar cells. Hematoxylin and eosin; reduced ⅙ from mag. × 350.

mitoses. They were surrounded by a delicate argyrophilic reticulum, which "faded out" toward the surface.

The focal inflammation was best developed in the loose, areolar visceral synovialis and varied from perivenular lymphocytic infiltration to well-defined, miliary, granulomatous lesions (Fig. 2). Some of these lesions were confluent, while others were spaced as far as 1 cm. apart, and their outlines conformed roughly to the drainage bed of one or more medium-sized venules. The lesions were composed of dense accumulations of lymphocytes, small mononuclear wandering cells, and proliferating fixed cells showing occasional mitoses. The lymphocytes extended centrifugally along venules for varying distances. The mononuclear wandering cells tended to have irregular, sausage-shaped nuclei and scanty, ill-defined cytoplasm. They were most numerous in the central portions of the lesions and seemed to be emigrating through the walls of certain venules and capillaries, which also showed endothelial hyperplasia with narrowing of the lumens (Fig. 3). Pyknosis and karyorrhexis of leucocytes were common within and outside the walls of such vessels, and some vessels were plugged by intraluminal collections of lymphocytes and monocytes (Fig. 4). Overlying the central portions of the miliary foci there were usually small patches of amorphous, fibrin-like material interspersed with a few pyknotic nuclei (Fig. 2). Occasionally there was also reticular fibrin radiating from inflamed vessels (Fig. 3). Intact neutrophiles were present in only a few focal lesions, where they emanated from superficial capillary loops. The synovial intima over the regions of intense exudation was frequently disrupted with scattering or necrosis of the synoviocytes (Fig. 5). There were also a few synoviocytic giant cells, with basophilic cytoplasm and up to four nuclei. In some instances, subintimal collagenous fibers were fragmented, fused, and sequestrated by the granulomatous tissue (Fig. 5). A few similar isolated collagenous fragments were also found in the absence of granulomatous inflammation, usually in association with minute intimal villi (Fig. 1).

In the subintimal fat, there were several foci of apparent atrophy with small fat vacuoles or foam cells. Deeper in the synovial membrane, inflammatory changes were minimal, but there was some patchy interstitial accumulation of an eosinophilic ground substance, which stained blue with anilin blue and replaced some of the fat cells. The fixed cells lying within this substance were often hypertrophied. A few fresh focal extravasations of red blood cells were probably the result of operative trauma.

Fig. 5 (Case 1; H-1879).—Synovial granuloma with necrobiosis. Note disruption and ischemia of superficial synovialis in passing from margin of granuloma, at the left, to its center, at right border of field. In the right upper corner there is an almost obliterated capillary surrounded by a few relatively well-preserved fixed cells. A sequestrated mass of fused collagenous fibers is seen to the right of center. Hematoxylin and eosin; reduced ½ from mag. × 180.



COMMENT

A three- to four-month episode of symmetrical polyarthritis involving the feet and knees began at an advanced age, was associated with mild constitutional symptoms, and subsided without permanent deformity. The synovial fluid was unusual in showing good mucin, slight leucocytosis, and a very high percentage of mononuclear cells.[12] Nevertheless, rheumatoid arthritis was thought to be a far more likely diagnosis than any other recognized disease entity. The principal microscopic features of the "seven-day" lesion were (1) slight diffuse intimal proliferation; (2) slight segmental infiltration of subintimal blood vessels and perivascular connective tissue by lymphocytes and small mononuclear wandering cells, occasionally associated with thrombosis or leucocytic plugging, and (3) superficial miliary granulomas characterized by infiltration of small polymorphic mononuclear wandering cells, disruption or necrobiosis || of the intima, fragmentation of collagenous bundles, and intensified vascular involvement with fibrin deposition. The segmental distribution and the intensity of the vascular changes relative to those in the adjacent tissue suggested the possibility of primary blood vessel damage.

CASE 2.—*Synovitis of right knee, nine days' "duration."*

H. D., a 59-year-old spinster, had been well most of her life except for occasional epistaxes, bouts of diarrhea, and "nervousness." At the age of 14 years, she had pain in her ankles, lasting several weeks. At 21 years, she had painful knees and hips for five weeks, but again recovered without residua. Three years before admission, the right knee was painful for several days, but physical examination was negative. Two months before entry, she began to have pain in her feet and had arch supports made. Ten days before entry, she began to have aching of the neck and shoulders with pain radiating from the left arm. These symptoms responded to local heat and acetylsalicylic acid. Four days before entry, during a spell of rainy weather, both knees became stiff and painful. By the following day, the hips and ankles were similarly involved, and she was unable to walk. On entry, she had a temperature of 101.4 F. and an upper respiratory infection with rhinorrhea. Both knees were moderately hot, tender, and swollen, with evidence of effusion. The ankles were tender, and there was tenderness over both greater trochanters and the metatarsal heads. The heart was normal. The hemoglobin was 13 gm. The white blood cell count was 10,600, with 83% neutrophiles. The corrected sedimentation rate (Rourke-Ernstene [11]) was 2.14 mm. per minute. X-ray films of the knees and feet showed no bony changes. The patient was treated with rest and acetylsalicylic acid, and, beginning one day after entry, there was rapid subsidence of the articular symptoms and the temperature. On the fifth hospital day, a biopsy of the right knee was performed. After the operation, the patient continued to improve slowly. After one month, all joints had normal range of motion, but slight soft-tissue thickening remained in both knees. The sedimentation rate was still elevated to 0.52 mm. per minute.

BIOPSY FINDINGS

Right Knee (#H-1929).—A medial parapatellar arthrotomy was performed, without the use of a tourniquet. The synovialis was slightly reddened but smooth. There was no pannus. A small effusion was present (Table 1).

Small pieces of visceral and parietal synovialis were fixed in formalin and in Zenker's fluid. Sections were stained with hematoxylin and eosin, phloxine-methylene blue, and toluidine blue (for metachromasia) and by the periodic acid-leucofuchsin technique. Frozen sections were examined for lipids. The predominant microscopic findings were a diffuse intimal proliferative reaction with scattered mitoses and palisading of the synoviocytes in some regions (Fig. 6) and some patchy surface deposits of fibrin-like material. Occasional coarse collagenous masses were present within or just beneath the intima. In the subintima ¶ there was slight to moderate fixed-cell proliferation and minimal perivascular infiltration of lymphocytes, mononuclear phagocytes, and mast cells. In the deep areolar portion of the visceral synovialis, there were several ill-defined foci of more intense inflammation, characterized by ede-

|| This denotes necrosis of a varying proportion of cells, with incomplete disintegration of the intercellular matrix.

¶ This term denotes the zone of richly vascular connective tissue immediately beneath the synovial intima. The subintima contains the most superficial arteriolar and venular plexuses and the capillary loops which arise from them.

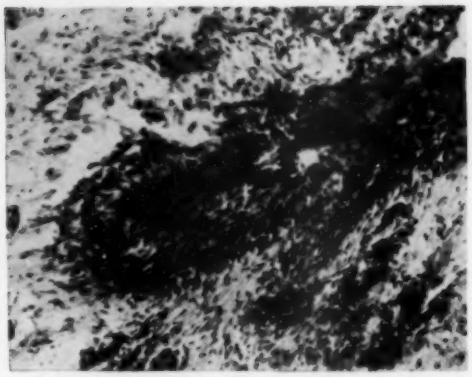


Fig. 6 (Case 2; H-1929).—Synovial recess showing palisading of proliferating synoviocytes and surface deposit of fibrin-like material. Hematoxylin and eosin; reduced ⅕ from mag. × 155.

ma, fibrin deposition, scattered neutrophiles, and proliferating polymorphic cells (Fig. 7). These cells tended to resemble Aschoff cells in having abundant basophilic cytoplasm and one to three hyperchromatic nuclei with central chromatin bars. Some arterioles in the vicinity of these regions contained fibrin thrombi. The edema-like interstitial substance was stained with about the same intensity as collagen by the periodic acid-leucofuchsin technique, but was not metachromatic. The fat stains (Sudan red) were negative.

Comment

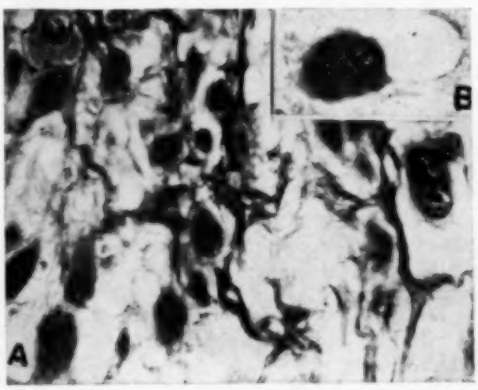
While the episodes of arthralgia in the history were all relatively mild and left no residua, such a course is not uncommon in mild rheumatoid arthritis. Moreover, the clinical features of the symmetrical polyarthritis which occasioned the patient's admission were entirely consistent with this diagnosis. The duration of involvement of the knee on which biopsy was performed was only eight days. However, the effusion had been present for at least five days and the articular symptoms had been subsiding for four days. The synovial fluid and anatomic findings had much in common with those in Case 1. Although the total white blood cell count in the synovial fluid was almost three times as high and the mucin precipitate was "fair," [13] there was the same striking predominance of mononuclear cells. Histologically, the proliferative intimal reaction was closely similar. No foci of angiitis or granulomatous inflammation were seen along the synovial surface, but the isolated collagenous masses and deeper-lying regions of edema and proliferating mesenchymatous cells resembled those seen in rheumatic and early rheumatoid nodules.#

Case 3.—*Synovitis of left knee, 10 days' to 6 weeks' "duration."*

D. B., a 2½-year-old girl, fell from a tricycle three months before admission. One week later she was noted to be limping and her left ankle was swollen. One month before admission, the patient was carefully examined by a private physician, and all other joints were normal. On entry, the rectal temperature was 99.4 F. The left ankle was swollen, indurated, warm, painful, and limited in motion. The left calf showed ½ in. of atrophy. The left knee was slightly swollen but had normal range of motion. The hemoglobin was 9.4 gm.; the white blood cell count was 7,150, with 55% neutrophiles, 38% lymphocytes, 4% monocytes, and 3% eosinophiles. A Hinton test was negative. The sedimentation rate was 55 mm. in one hour (Westergren). X-ray films of the left knee and ankle showed only soft-tissue thickening. On a program of bed rest, local heat, and acetylsalicylic acid, tenderness and warmth of the involved joints decreased, but on the eighth hospital day an effusion appeared in the left knee. After biopsy of the left knee, on the 10th hospital day, the knee and ankle gradually improved,

Reference 4 and unpublished observations.

Fig. 7 (Case 2; H-1929).—(*A*) High-power field from localized region of edema, reticular fibrin deposition, and proliferating mesenchymatous cells. Note mitosis in upper left-hand corner and "owl eye" cells. PAS and hematoxylin; reduced ⅛ from mag. × 390. (*B*) Trinucleated "Aschoff" cell from adjacent field; PAS and hematoxylin; reduced ⅕ from mag. × 390.



but within the next 4 months the left thumb, right fourth toe, and right knee became involved. Mild limitation of motion and elevation of the sedimentation rate continued for nine months after entry and then gradually subsided. Fifteen months after entry, only the left ankle was limited in motion.

Biopsy Findings

Left Knee (#H-1876).—The left knee was opened through a medial parapatellar incision, without the use of a tourniquet. About 5 cc. of clear straw-colored fluid was present in the joint cavity (Table 1). The synovialis was moderately hyperemic and hypertrophied with some large villi. The cartilaginous surfaces of the medial femoral and tibial condyles appeared normal. A small piece of fibrous parietal synovialis just medial to the medial femoral condyle was removed.

Fig. 8. (Case 3; H-1876).—Capsular synovialis with intimal and subintimal hyperplasia, palisading of superficial synoviocytes, and fibrinopurulent exudate on surface; hematoxylin and eosin; reduced ⅙ from mag. × 220.

Microscopic examination revealed a diffuse, nonsuppurative inflammation, which varied somewhat in intensity and was confined almost entirely to the intima and subintima (Fig. 8). In regions of more marked inflammation, there were thin patches of fibrinous surface exudate admixed with a few neutrophiles. The intima varied from two to seven apparent cell layers in thickness. The synoviocytes tended to be rounded with ill-defined cell outlines and resembled the mononuclear cells in the exudate, but in places beneath the surface exudate they were arranged in palisade formation (Fig. 8). The basal synoviocytes were surrounded by a delicate argyrophilic reticulum. The subintima was hyperemic, with apparent increase in vascularity, and contained scattered lymphocytes, mononuclear wandering cells, and rare neutrophiles. In one region, there was edema, possibly of traumatic origin.

Comment

The diagnosis of rheumatoid arthritis was confirmed by the development of chronic polyarthritis with symmetrical involvement of the knees. As in Case 1, the effusion was of two days' duration when aspirated. The fluid did not clot; the mucin precipitate was "fair," [12] and the white blood cells were more numerous. Again, these cells were almost entirely mononuclear. The biopsy specimen of fibrous parietal synovialis showed superficial, proliferative synovitis, with some surface deposits of fibrin and palisading of synoviocytes, but no obvious vascular damage. It should be noted that in the previous two cases vascular lesions were found only in the visceral synovialis.

Case 4.—*Synovitis of left knee, 16 days' to 3½ months' "duration," and communicating popliteal cyst, of 3½ months' "duration."*

B. O., a 60-year-old single domestic, noted swelling, stiffness, and pain in the left knee three and one-half months before entry. With bed rest the symptoms disappeared in a few days, but swelling in the left popliteal fossa persisted. One week before entry, the left knee again became stiff, painful, and swollen; three days later, the right knee was similarly affected. On admission, the vital signs were normal. The left knee was warm, tender, and had a moderate effusion. A cyst measuring 6 by 3 by 2 cm. was present in the popliteal space. The right knee was swollen and tender. The hemoglobin was 12 gm.; white blood cell count was 5,500, with 80% neutrophiles and 18% lymphocytes. The sedimentation rate (Wintrobe, uncorrected) was 30 mm. in one hour. X-ray films of the knees showed free fluid, soft-tissue thickening, and some degenerative joint disease, but the bones were well mineralized. Aspiration of the left knee resulted in 50 cc. of cloudy yellow fluid (Table 1). The patient improved under conservative treatment. Nine days after ad-

mission, when the left knee was still warm and slightly swollen and the sedimentation rate was 1.35 mm. per minute (Rourke-Ernstene [11]), the left popliteal cyst was excised and a synovial biopsy of the left quadriceps pouch was performed. After operation the patient continued to improve, but shortly after discharge she again developed pain and effusions in both knees, which gradually led to complete incapacity.

Biopsy Findings

Left Popliteal Cyst (#H-1815A).—At operation the cyst was found to communicate with the knee joint through an ostium 1.0 cm. in diameter. The cyst, as well as the joint, contained a small amount of thin, slightly cloudy fluid with a few flecks of fibrin. The cyst lining was very shaggy with many soft, "somewhat sticky" tags. The exposed cartilage was yellowish and roughened.

On microscopic examination, the cyst wall was composed of moderately dense connective tissue, averaging 3 mm. in thickness. The lining was ragged, intensely inflamed, and covered by adherent tags of fibrinopurulent exudate and more or less devitalized tissue. Near the opening into the joint, the cyst lining was continuous with hypertrophic synovialis.

The exudate within the cyst consisted of a coarse, fibrin-like reticulum, in the meshes of which there were varying numbers of neutrophiles and various mononuclear cells, including foamy histiocytes and large polymorphic cells with hyperchromatic nuclei and basophilic cytoplasm. Some of these inflammatory cells were undergoing necrosis. There were also occasional isolated fragments of collagenous tissue and cartilage, most of which contained some viable cells. The inflammatory reaction in the cyst lining was varied. In focal regions, the fibrin-like substance and inflammatory cells infiltrated the superficial tissue to varying depths, accumulating along cleavage planes between the collagenous bundles and appearing to split bits of dense connective tissue (Fig. 9). Elsewhere there was intense proliferation of fibroblasts and capillaries, with some invasion of the exudate. Small hemorrhagic extravasations were common in the zone immediately beneath the exudate, and occasional hemosiderin granules were present in the deeper tissues. The intact portions of synovial membrane showed slight villous hypertrophy and marked proliferation of the intima, which varied up to five apparent cell layers in thickness. Cellular infiltration in the subintima varied from minimal to moderate, with lymphocytes predominating in some areas and neutrophiles in others. There were occasional foreign body giant cells. Hypertrophy and hyperplasia of fixed cells and small vessels were generally prominent. The superficial vessels were thickened and narrowed by the proliferative process, and some contained fibrin thrombi. The deeper portions of the cyst wall showed slight edema, fibrosis, and perivascular lymphocytic infiltration.

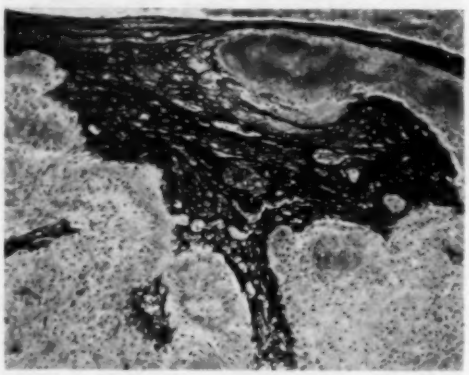


Fig. 9 (Case 4; H-1815A).—Lining of popliteal cyst showing interstitial deposition of fibrin-like material associated with necrobiosis and connective tissue fragmentation. Phosphotungstic acid hematoxylin; reduced ½ from mag. × 115.

Left Knee (#H-1815C).—An incision was made along the anteromedial aspect of the quadriceps pouch. The synovialis appeared slightly edematous but was not strikingly injected or hypertrophic. The cartilage of the medial femoral condyle was normal, and no pannus was seen.

The biopsy specimen consisted of fatty synovialis, which showed moderate intimal hyperplasia and fibrosis but little exudative inflammation. The intima varied up to four apparent cell layers in thickness. Inflammatory cell infiltration was mainly subintimal and consisted of scattered lymphocytes, with occasional histiocytes and rare neutrophiles.

The fibrosis was patchy and involved both the subintima and the adjacent connective tissue septa. The fibroblasts in the fibrotic regions were plump and often had prominent cytoplasmic processes. One superficial patch of fibrosis was circumscribed and partly hyalinized (Fig. 10). There was also a focus of fat injury, with infiltration of foam cells.

COMMENT

The diagnosis of rheumatoid arthritis was based mainly on the recurrent symmetrical involvement of the knees. The initial symptoms, 3½ months before biopsy, lasted for only a few days and did not recur until 16 days before operation, but the inflammatory

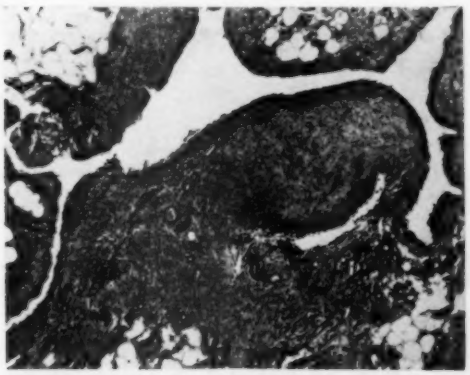


Fig. 10 (Case 4; H-1815C).—Nonspecific chronic synovitis and patchy fibrosis in patellar fat pad; hematoxylin and eosin; reduced ½ from mag. × 94.

popliteal cyst, which developed at the onset, persisted. At the time of biopsy, the joint had been subsiding again for eight days but was still warm and slightly swollen. The popliteal cyst, which communicated with the joint cavity, resembled a rheumatoid nodule in showing central necrobiosis, fragmentation of collagenous tissue, and accumulation of fibrin-like substance surrounded by a zone of nonsuppurative or granulomatous inflammation. The vessels in this zone were sparse or narrowed by endothelial hypertrophy, and there was evidence of recent and old focal hemorrhage. The low degree of inflammatory activity in the anterior portion of the joint was consistent with the subsidence of clinical signs during the preceding eight days. The patchy distribution of the scarring suggested a localized form of tissue injury.

CASE 5.—*Synovitis of right knee, 4½ months' "duration," but subsiding for 20 days.*

L. H., a 19-year-old housewife, had had three previous episodes of arthritis: At the age of 15 months the left knee was involved for six months; at 5 years both knees were affected for four months and there was an associated iridocyclitis of the left eye; and at 10 years both knees and the right wrist were involved for one year. She then remained asymptomatic until four months before entry, when she again developed pain, stiffness, and swelling of both knees. One week later, the elbows became involved, followed in three weeks by the ankles and fingers. Five days prior to entry, she complained of pain in the back, the second right proximal interphalangeal joint, and all the metatarsophalangeal joints. On admission, the vital signs were normal. The knees showed moderate effusions and periarticular thickening. The right knee was warmer than the left and showed 5 degrees of permanent flexion, with further flexion to 100 degrees. The left knee showed full extension, with further flexion to 120 degrees. The metatarsophalangeal joints were tender. The elbows showed soft-tissue thickening and 15 degrees of permanent flexion. The second left metacarpophalangeal joint was swollen, and both wrists were painful on motion. The hemoglobin was 11 gm.; the white blood cell and differential counts were normal. The sedimentation rate (Rourke-Ernstene[11]) was 1.25 mm. per minute. X-ray films of the knees showed soft-tissue thickening. In the hospital, the patient improved on conservative treatment. Twenty days after entry, a biopsy of the right knee was performed. At this time the sedimentation rate had fallen to 1.07 mm. per minute, and the joint effusions had subsided. One year later, the sedimentation rate was 0.47 mm. per minute, but there was no evidence of joint disease.

BIOPSY FINDINGS

Right Knee (#H-1648).—At operation the right knee joint was found to contain no appreciable amount of fluid. The synovialis was thickened and reddish-purple. No pannus was apparent.

Microscopic examination of a section from the suprapatellar pouch showed moderate villous hypertrophy, with intense fixed-cell hyperplasia, marked infiltration of lymphocytes and plasma cells, and a few neutrophiles. Lymphocytic nodules were present within some of the villi. The intima was hyperplastic, averaging about six apparent cell layers in thickness, and frequently showed

palisading of the hypertrophied synoviocytes (Fig. 11). Mitoses and binucleate cells were not uncommon. All but the most superficial synoviocytes were surounded by delicate reticulum fibers. Along the surface some of the cells appeared to have undergone karyolysis. In several synovial recesses there was slight interstitial deposition of fibrin and a small patch of fibrinopurulent exudate was adherent to the intima (Fig. 11). The minute subintimal blood vessels were narrowed by cellular proliferation and hypertrophy, resulting in apparent ischemia of the superficial hyperplastic tissue. The larger, deeper-lying venules and veins were congested. In places there was slight edema. Slight focal hemosiderin deposition was present. In the deeper synovial connective tissue, the inflammation was less marked and almost entirely juxtavascular.

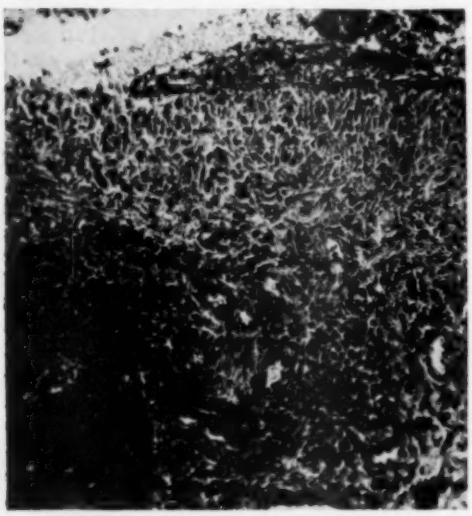


Fig. 11 (Case 5; H-1648).—Rheumatoid synovitis showing adherent patch of fibrinopurulent exudate, intense intimal hyperplasia with palisading of the superficial synoviocytes, and a lymphoid nodule. Note the relative avascularity of the hyperplastic superficial zone. Hematoxylin and eosin stain; reduced ⅓ from mag. × 170.

Comment

This patient had typical symmetrical rheumatoid polyarthritis. The previous attacks in childhood were probably too remote to contribute significantly to the inflammatory features of the four-and-a-half-month-old lesion on which biopsy was performed. Although the inflammatory process in the joints examined had been subsiding clinically for at least 20 days, the presence of fibrinopurulent surface exudate and the intense synoviocytic proliferation suggested the continuance of inflammation. The synovitis was of the "classic" rheumatoid type, with hypertrophied villi containing lymphocytic nodules. The minute superficial vessels were narrowed by endothelial hypertrophy. Thus, the purplish gross appearance of the synovialis was presumably caused by engorgement of the underlying venous plexus.

Case 6.—*Synovial and semilunar cartilage lesions of left knee, five months' "duration."*

E. T., a 21-year-old single woman, first noted pain, stiffness, and swelling of the left knee five months before entry. The symptoms became progressively worse, and she lost 15 lb. (6.8 kg.) in weight. On admission, the vital signs were normal. The left knee was warm and moderately swollen, with permanent flexion of 20 degrees and further flexion to 80 degrees. The hemoglobin was 80%; white blood cell count was 7,800. X-ray films of the left knee showed some joint narrowing and "bone atrophy." Four days after entry, when the knee was still painful and limited in motion but less swollen, a partial synovectomy and meniscectomy were performed. Five months after entry, she began to have pain and swelling of the right knee, as well as pain and stiffness of the neck, left elbow, and left ankle. She gradually recovered and remained well for 16 years, when she had another 3-month episode of pain, stiffness, and swelling of the right knee and left shoulder.

Biopsy Findings

Left Knee (#H-60).—The operation was performed with a tourniquet applied. The joint contained about 2 cc. of amber fluid. The synovialis was greatly thickened, redundant, and bluish to grayish red. Some areas were suggestive of "caseous deposits." The cartilaginous surface of the patella was almost entirely covered with pannus. The condyles of the femur and tibia showed synovial adhesions. Along the medial margin of the lateral tibial condyle, the cartilage was destroyed down to bone. The medial meniscus resembled a fibrous shred, and the anterior margin of the lateral meniscus was largely replaced by fibrous tissue.

Microscopic examination of sections from the quadriceps pouch, the infrapatellar fat pad, and both menisci showed a chronic synovitis with hypertrophic villi and folds, separated in places by deep crypts. The intima was generally hyperplastic, with hypertrophy of the synoviocytes, which at times were arranged in palisade formation with up to six apparent cell layers. The exudative inflammatory reaction was characterized by scattered, discrete, lymphocytic nodules, as well as diffuse and perivascular infiltration of lymphocytes and plasma cells. Many of the lymphocytic nodules were situated within the villi, and some had well-developed reaction centers. Occasional Russell bodies were associated with the plasma cells. There was

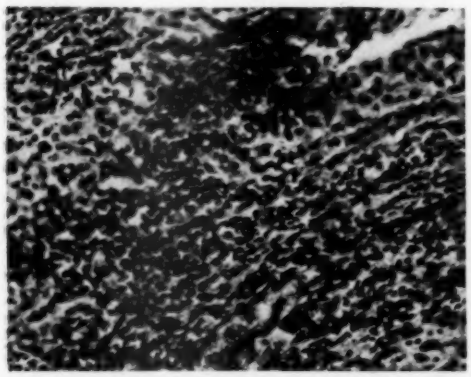


Fig. 12 (Case 6; H-60).—Focal neutrophile infiltration adjacent to synovial crypt (upper right); hematoxylin and eosin; reduced ½ from mag. × 300.

also a scattering of neutrophiles, eosinophiles, and macrophages, and several juxtavascular collections of neutrophiles were present adjacent to a synovial crypt in the quadriceps pouch (Fig. 12). In the infrapatellar fat pad there were a number of patchy superficial deposits of an amorphous, fibrin-like material which resembled an organizing fibrinous exudate (Fig. 13), but in places this material permeated the synovial lining and obscured the collagenous stroma (Fig. 13). Occasionally such fibrin-like regions were partly detached, forming semidetached fringes. Fixed-cell hypertrophy and proliferation were common throughout the subintima and extended to

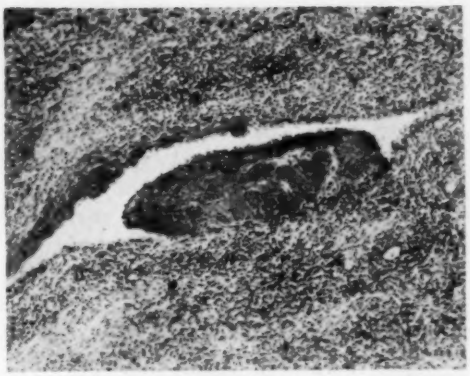
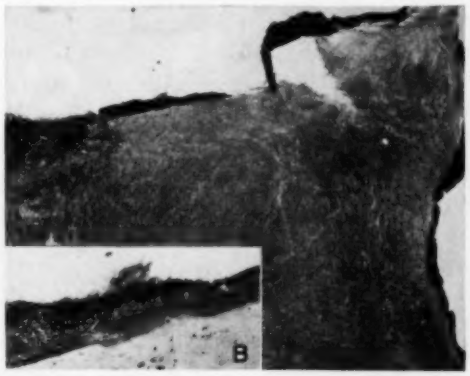


Fig. 13 (Case 6; H-60).—Synovial crypt from infrapatellar fat pad showing chronic inflammation and fibrin-like deposits. The large deposit on the lower surface appears to represent a patch of organizing surface exudate, while the more diffuse deposit on the upper surface seems to have formed interstitially. In both instances the fixed cells, which are completely surrounded by the fibrin-like matrix, tend to be atrophic. Hematoxylin and eosin; reduced ½ from mag. × 115.

varying degrees into the deeper tissues. In places there was fibrosis or interstitial accumulation of slightly hematoxylinophilic, "mucinous" ground substance. In one section there were large slit-like spaces lined by a single layer of cells and containing a protein-rich fluid with occasional lymphocytes. Edema was generally slight or absent. There were a few small hemosiderin deposits.

Fig. 14 (Case 6; H-60).—(*A*) Medial meniscus with pannus encroachment at synovial margin and formation of fibrin-like material along the free surfaces; hematoxylin and eosin stain; reduced ⅓ from mag. × 25. (*B*) Higher magnification of advance margin of pannus. Interstitial fibrin-like material and fragmented nuclei are seen in the superficial cartilage distal to the pannus, as well as in the pannus itself. Reduced ½ from mag. × 115.



Many small blood vessels were prominently involved by the general fixed-cell reaction, with thickening of their walls and narrowing of the lumens. Such vascular proliferation and narrowing were particularly prominent in the subintima, which thus appeared relatively ischemic (Fig. 13). The deeper veins were often congested. Some large arterioles were partly or completely obliterated by fibrosis.

The medial meniscus was covered over the peripheral half of each surface by an ingrowth of synovial pannus (Fig. 14). The remaining portions of the free surfaces showed a narrow zone of at least partly interstitial, fibrin-like substance, associated with some necrobiosis and disruption of the cartilage matrix and with infiltration of scattered mononuclear cells and neutrophiles (Fig. 14). The lateral meniscus showed essentially similar changes.

Comment

The onset with monarticular arthritis and the gross findings at operation were somewhat suggestive of tuberculous arthritis, but the clinical course over a period of 17 years, with two episodes of polyarthritis and subsequent remissions, was consistent with rheumatoid arthritis. Biopsy of the knee was performed when the lesion had already subsided slightly, as indicated by a reduction in articular swelling, but four days before operation the joint had still been warm, severely painful, and enlarged. The microscopic findings included, in addition to the "classic" features of rheumatoid synovitis, patchy deposits of fibrin-like material, which presumably corresponded to the "caseous deposits" noted grossly. The involvement of cartilage by these changes in advance of the pannus is of particular interest. The slit-like spaces containing protein-rich fluid were an unusual finding. They might possibly have represented synovial crypts which had become isolated. Vascular changes consisted mostly of hypertrophic thickening and narrowing, which might have limited the blood supply to the superficial synovialis.

Case 7.*—*Right popliteal cyst, seven months' "duration," and synovitis of the same knee, nine months' "duration."*

L. H., a 16-year-old girl, developed aching in both feet one year before entry. Six months before entry, she noted the onset of pain and swelling of the right knee and a soft swelling in the right popliteal fossa. Two months later, she began to have pains in both shoulders and stiffness, pain, and swelling of both wrists. She also noticed some loss of weight and increased fatigability. On admission, there was slight limitation of motion in both wrists and swelling of the right knee, with a cystic mass in the popliteal fossa. The hemoglobin was 12.7 gm.; white blood cell count was 7,800, with a normal differential count. The sedimentation rate (Rourke-Ernstene [11]) was 0.80 mm. per minute. X-ray films of the knees appeared normal. The hands showed soft tissue swelling of the metacarpophalangeal and proximal interphalangeal joints. In the hospital, the patient ran temperatures up to 100.0 F., and the sedimentation rate remained elevated. Three weeks after entry, the right popliteal cyst was removed. The knee continued to be swollen and painful. Three months after entry, an anterior arthrotomy was performed. The sedimentation rate at that time was 0.75 mm. per minute. The patient developed fusiform swelling of the fingers, limitation of motion in both wrists and left elbow, and a right olecranon bursitis. A histologically typical rheumatoid nodule was removed from the left great toe. When last seen, nine years after discharge, the patient appeared well but had some pain in the shoulders and knees.

Biopsy Findings

Right Popliteal Cyst (#H-1044A).—At operation the cyst wall appeared to blend with the capsule of the knee joint. The sac contained a few milliliters of clear fluid and masses of a gelatinous material.

On microscopic examination the cyst wall was seen to be composed of dense fibrous tissue with a ragged, inflamed synovial lining. This lining showed widespread, patchy, caseous necrosis (Fig. 15) and occasional bits of fibrin-like material. In regions where the synovial intima was preserved, the synoviocytes were loosely knit, rounded, hypertrophic, and intermingled with chronic inflammatory cells. The subintima was heavily

* The Robert Breck Brigham Hospital permitted inclusion of this case, and Dr. Philip LeCompte, pathologist at the Faulkner Hospital, allowed us to study the biopsy specimen from the popliteal cyst.

infiltrated by lymphocytes and polymorphic, histiocyte-like cells. Some of these cells had pyknotic nuclei and eosinophilic hyaline inclusions. Neutrophiles were rare, even adjacent to regions of necrosis. Scattered throughout the cyst wall, there were some perivascular and large nodular aggregates of lymphocytes, plasma cells, and histiocytes, extravasations of red blood cells, and small hemosiderin deposits. The minute blood vessels in some regions showed proliferative thickening of their walls and narrowing of the lumens, and adjacent to the necrotic lining, many contained fibrin thrombi (Fig. 15). In other regions there was intense hyperemia.

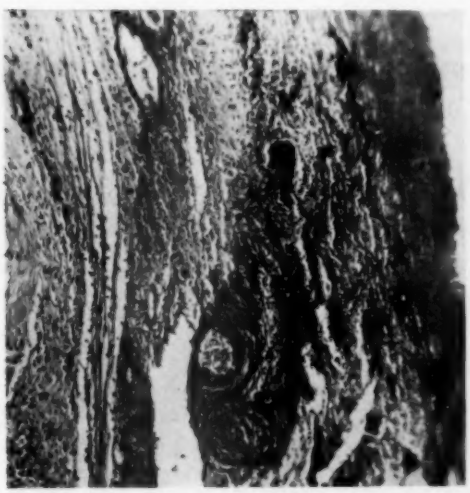


Fig. 15 (Case 7; H-1044A).—Popliteal cyst wall with caseous necrosis of lining and thrombosis of adjacent blood vessels; hematoxylin and eosin stain; reduced ⅙ from mag. × 115.

Right Knee (#H-1044B).—On anterior exposure, the joint showed 20 to 30 cc. of clear mucinous fluid; soft, friable, fibrin-like loose bodies; "soft, filmy synovial adhesions," and pannus formation. The synovial membrane appeared thickened, hyperemic, and edematous.

On microscopic examination, the loose bodies consisted of necrotic, fibrinopurulent exudate, blending with amorphous fibrin-like material which contained scattered viable fixed cells and was bordered in places by an intact layer of synoviocytes. The synovial membrane was characterized by villous hypertrophy and lymphocytic nodules, which tended to be located within the villi (Fig. 16). Occasional small patches of fibrinous exudate were adherent to the surface. The intima was hyperplastic, varying up to six apparent cell layers in thickness. Moreover, in some fissure-like synovial crypts there was palisading of the synoviocytes, occasional giant cell formation, and an amorphous blending of the superficial cell bodies suggestive of necrobiosis (Fig. 17). The subintima showed moderate, predominately perivascular infiltration of lymphocytes, plasma

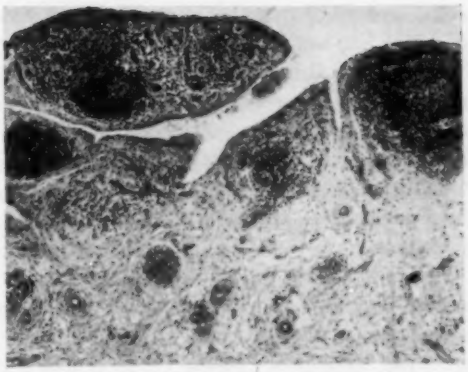
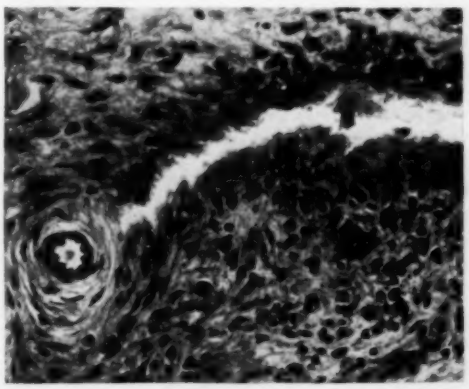


Fig. 16 (Case 7; H-1044B).—Classic rheumatoid synovitis with villous hypertrophy and lymphocytic nodules. Note reaction center in nodule at right and thickening of blood vessels with round cell infiltration. Hematoxylin and eosin stain; reduced ½ from mag. × 60.

Fig. 17 (Case 7; H-1044B).—Superficial granulomatous reaction in fissure-like synovial crypt; hematoxylin and eosin stain; reduced ⅙ from mag. × 390.



cells, and many lymphocytic nodules, some of which had well-developed reaction centers. There were also scattered histiocytes and neutrophiles. Numerous Russell bodies were associated with some collections of plasma cells. The fixed cells were moderately hypertrophied. The superficial blood vessels were hyperemic, and some were thickened by proliferation, fibrosis, and round cell infiltration (Fig. 16). There was some focal extravasation of red blood cells and perivascular hemosiderin deposition. There was also a considerable increase in granular, slightly eosinophilic interstitial substance, with separation of the tissue elements. It was not established whether this substance was edema or ground substance. In the deeper synovial areolar tissue this "edema" was even more striking, while cellular infiltration was relatively slight.

Comment

This patient resembled Case 4, in that she developed an inflammatory popliteal cyst with the onset of joint involvement. In contrast to Case 4, fibrin-like material within the cyst was scanty. Instead, the inflammation was characterized by caseous necrosis, associated with thrombosis of adjacent blood vessels and histiocytic infiltration. The synovitis in the anterior portion of the joint was of the "classic" type, but in some crypts there was a laminar granulomatous reaction, somewhat suggestive of that in rheumatoid nodules (Fig. 17).

Case 8.—*Synovitis of left knee, nine months' "duration," but subsiding for three months and asymptomatic at time of biopsy.*

A. Q., a 13-year-old girl, first noted pain and swelling in her right ankle four and a half months before entry, but within two days it returned to normal. Four months before entry, she developed migratory pains in the neck, elbows, wrists, interphalangeal joints, knees, and ankles. No joint was symptomatic for longer than a week. There was also a recurrent, generalized, violaceous, macular rash and occasional afternoon chills. Menstruation ceased, and she lost 12 lb. (5.4 kg.) during the next three months. On admission, the temperature was 104 F. and pulse 130 per minute. There was generalized lymphadenopathy and slight splenomegaly. The heart was normal in size; there was a Grade I apical systolic murmur. The wrists, fingers, and right ankle were painful on motion. There was moderate spindling of the fingers. Both knees showed synovial thickening and small effusions, but normal range of motion. The hemoglobin was 10 gm.; white blood cell count was 6,000, with 88% neutrophiles and 10% lymphocytes. The sedimentation rate (Rourke-Ernstene [11]) was 1.37 mm. per minute. X-ray films of the knees showed no bony changes. On aspiration of the left knee, 24 cc. of turbid yellow fluid was obtained (Table 1). During the first two months in the hospital, the patient had intermittent fever, but she gradually improved. A biopsy of the left knee was performed five months after admission, at which time the patient was essentially asymptomatic. However, the left knee still showed some soft tissue thickening and tenderness over the joint line. The sedimentation rate was 1.22 mm. per minute. After the operation the patient went into complete remission, but two years later there was a recurrence of symmetrical polyarthritis, which left residual flexion deformities of both hips, with narrowing of the joint spaces as seen by x-rays. Four years later the patient was still in remission. The knees had full range of motion but showed slight soft-tissue swelling.

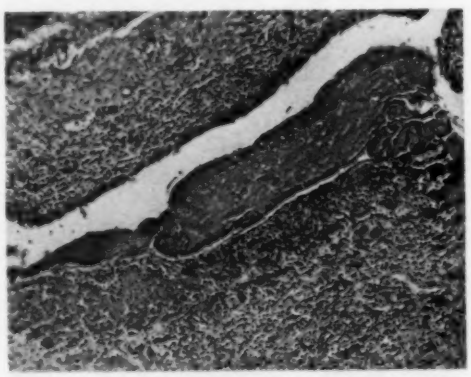


Fig. 18 (Case 8; H-1558).—Interstitial and superficial deposits of fibrin-like material. Note relatively scant number and atrophic appearance of cells within the fibrin-like matrix. Hematoxylin and eosin; reduced ½ from mag. × 115.

Biopsy Findings

Left Knee (#H-1558).—At operation, the capsular tissues were seen to be markedly thickened and the synovial membrane inflamed and friable. The joint fluid was moderately increased in amount. Some semiloose, soft, friable white fringes were present in the suprapatellar pouch. The margins of the patella were covered with pannus.

Microscopic examination of synovialis from the suprapatellar pouch showed a dif-

fuse, chronic inflammation on which were superimposed patchy superficial and interstitial deposits of fibrin-like material (Fig. 18). Some of these patches corresponded to the semiloose white fringes noted grossly. Within the fibrin-like matrix there were scattered, viable and necrotic macrophages, neutrophiles, and fragments of collagenous tissue. Some of the mononuclear cells contained droplets of birefringent lipid. There was also some overgrowth of the fibrin-like masses by synoviocytes and invasion by fibroblasts, which were surrounded by reticulum fibers. Capillary ingrowth, however, was rare. Where the fibrin-like material permeated the synovialis, the intimal architecture and underlying collagenous fibers were obscured.

In other regions the synovialis showed marked hypertrophy and hyperplasia of all types of fixed cells, with many mitoses. In the subintima there was also moderate diffuse and perivascular infiltration of lymphocytes; a scattering of plasma cells, neutrophiles, and eosinophiles; considerable perivascular hemosiderin deposition, and fibrosis. In places the adipose tissue showed irregular reduction in the size of the vacuoles and scattered foam cells. The minute subintimal vessels were markedly involved by the general proliferative process, with increase in their number and narrowing or obliteration of the lumens. A few venules contained plugs of mononuclear cells. There was little or no edema.

Comment

This case was unusual in the severity of the initial constitutional reaction, but the subsequent recurrence of symmetrical polyarthritis and development of permanent joint deformities confirmed the diagnosis of rheumatoid arthritis. The fibrin-like deposits were the most striking feature of the articular lesion. The abundance of these deposits and their limited penetration by connective tissue ingrowth, despite the clinical decline of the lesion during the preceding three months, suggest the possibility that the fibrin-like material is peculiarly resistant to both resolution and organization. The synovial fluid findings in the cases presented are shown in Table 1. The three effusions of under 10 days' duration which were associated with the three earliest lesions were remarkable for a striking preponderance of mononuclear phagocytes and a relatively slight degradation of mucin, as compared with that in rheumatoid effusions of longer standing. It is not clear whether these features reflect the brief duration of the lesions or the relatively mild form of the disease. Among 16 other rheumatoid effusions of nine or less days' duration studied, only 3 had less than 30% neutrophiles. Nine of these fluids were examined for mucin: It was good in two, fair in three, poor in two, and very poor in two.

Table 1.—*Synovial Fluid Findings*

Case No.	Duration of Effusion, Days	Amount Aspirated, Ml.	Gross Appearance	Total RBC per Cu. Mm.	Total WBC per Cu. Mm.	Per Cent Cell Types * P	L	M	Mucin[12]	Miscellaneous
1	2	30	Turbid yellow	1,000	550	4	6	90	Good	Clotted rapidly
2	5-9	5	Slightly turbid yellow	.....	1,650	0	12	88	Fair	Clotted rapidly
3	2	5	Clear, straw-colored	9,300	14,550	0	10	90	Fair	No clot; viscosity 10.4 at 38.6 C.
4	16	50	Thin, cloudy, flecks of fibrin	.....	.....	..	..	..	Poor	Sugar difference † 21 mg./100 ml.
5	No Joint Aspiration Performed									
6	(?) 150	2	Amber	.....	.....	..	..	..	.....	.....
7	(?) 200	30	Clear, mucinous	.....	.....	..	..	..	.....	.....
8	(?) 120	25	Turbid yellow	400	62,200	91	4	5	Poor	Viscosity 9.7 at 38 C.; sugar difference, 15 mg./100 ml.

* P, polymorphonuclear leucocytes; L, lymphocytes; M, mononuclear phagocytes.
† Sugar difference = difference between the concentrations of glucose in the serum and in the synovial fluid.

TABLE 2.—*Biopsy Findings*

Case No.	Lesion Examined	Clinical Duration of Lesion	Period of Subsidence Before Biopsy	Gross Appearance of Synovialis	Pannus	Villous Hypertrophy	Intimal Hypertrophy and Hyperplasia	Fixed Cell Proliferation	Edema or Increased Ground Substance	Lymphocyte Infiltration	Lymphocytic Nodules	Plasma Cell Infiltration	Neutrophile Infiltration	Fibrin or Fibrin-like Material	Necrobiosis	Hemorrhage	Vascular Narrowing
1	Knee	7 days	0	Smooth, slightly hyperemic	0	±	+	+	+*	+	0	0	+*	+	+	±	++†
2	Knee	9 days	4 days	Smooth, slightly hyperemic	0	±	++	+	++*	+	0	0	+*	++	0	0	0†
3	Knee	10-42 days	0	Moderately hypertrophic, hyperemic	0	+	++	+	0	+	0	0	+	++	±	±	0
4	Popliteal cyst	3½ mo.	(?) 8 days	Shaggy with synovial tags	—	±	+++	+++	++	++	0	0	+++*	+++	++	+‡	+
	Knee	½-3½ mo.	8 days	Slightly "edematous"	0	0	++	+	0	+	0	0	+	0	0	0	±
5	Knee	4½ mo.	20 days	Thick, purple	0	+	+++	+++	+	+++	+	+++	+	+	±	0‡	++
6	Knee	5 mo.	4 days	Thick, purple, "caseous deposits"	+++	++	+++	+	++*	+++	+§	+++	++*	++	+	0‡	+
7	Popliteal cyst	7 mo.	(?) 0	Contents: clear fluid and gelatinous material	—	0	+	+*	+	++	+	+	+	+	+++	+‡	++
	Knee	9 mo.	(?) 0	Hyperemic, "edematous," fibrin-like loose bodies, and filmy adhesions	+	++	+++	+	++	++	++§	++	+	+	±	+‡	++†
8	Knee	9 mo.	3 mo.	Inflamed, "semiloose, white fringes"	+	+	++	++	±	++	±	0	+	+++	+	+‡	++†

* Localized.
† Thrombi.
‡ Hemosiderin.
§ Reaction centers.

However, most of these fluids were from joints which either had been symptomatic for some time before the onset of effusions or had been involved in the recent past.

The anatomic findings of the eight biopsies of the anterior knee joint and the two popliteal cysts are summarized in Table 2. In view of the regional variations within the same joint and consequent magnitude of sampling error, the quantitative estimates of the microscopic changes must be regarded only as impressions. Comparison of the lesions is further complicated by the variations in their severity and the fact that some were at the peak of their clinical activity while others had passed their peak by varying periods of time.

GENERAL COMMENT

The principal criterion in selecting the cases for this study was the presence of chronic symmetrical polyarthritis in the absence of known specific etiologic factors. Thus, we obtained a series which falls within the present clinical concept of rheumatoid arthritis.[10] At least an equal number of cases with probable rheumatoid arthritis were rejected because they did not meet our standard.

Despite the attempt to limit our case material to a reasonably homogeneous group, the variability of the lesions made it difficult, if not impossible, to reconstruct their development. However, through the study of these early and relatively active joint lesions, the arthritic process becomes more intelligible as a manifestation of the same basic tissue reactions which characterize rheumatoid disease throughout the body. These reactions, which vary to some extent independently of each other, will be discussed under the following headings: (*a*) generalized proliferative inflammation, (*b*) formation of fibrin or fibrin-like deposits and necrobiosis, and (*c*) vascular lesions.

Generalized Proliferative Synovitis.—The proliferative inflammatory process was generalized throughout the synovialis but tended to be particularly pronounced in portions adjacent to articular cartilage. Microscopically, it was present in every instance, but grossly the two lesions of less than 10 days' "duration" showed only slight hyperemia. "Edematous" thickening and hypertrophy of villi were first obvious in the "10- to 42-day" lesion. Pannus formation with synovial tissue growing out over and replacing articular cartilage was well established in the lesions of five months' "duration" or more.

Intimal hyperplasia with occasional mitoses and stratification or pseudostratification of synoviocytes was present even in the earliest lesions. In relatively protected portions of the joint lining, the more superficial cells often extended cytoplasmic processes toward the lumen and became arranged in palisade formation (Fig. 8). The deeper cells were generally surrounded by delicate argyrophilic reticulum fibers, as has also been demonstrated in the synovitis of disseminated lupus erythematosus.[18] Occasional plump superficial cells with basophilic cytoplasm contained two or more nuclei. Generalized subintimal fixed-cell proliferation was not prominent until the "10- to 42-day" lesion, coinciding with the appearance of villous hypertrophy. Maximal cellularity of the superficial synovialis was reached in the "four and a half month" lesion (Fig. 11). True edema was prominent only occasionally in localized regions. In some instances, however, the areolar synovial tissue showed an increase in mucinous ground substance (Fig. 2), which may have contributed to the "edematous" gross appearance. Widespread fibrosis was not prominent in these early lesions.

Inflammatory cell infiltration tended to be focal and juxtavascular. It was relatively mild in the earliest lesions and was least well developed where the tissue was dense and fibrous. Lymphocytes were generally the most numerous, but neutrophiles and mononuclear wandering cells were not uncommon and occasionally predominated in focal regions. The "classic" rheumatoid synovitis, with lymphocytic nodules and villous hypertrophy (Fig. 16), was present only in three

lesions, all of which were of more than four months' "duration."

The specificity of the generalized synovitis is still a matter of debate. Experimentally, a diffuse proliferative reaction, with villous hypertrophy and pannus formation, has been elicited by intra-articular injection of various nonspecific irritants, including India ink and homologous blood.[14] However, Collins[15] maintains that "very large lymphoid foci and follicles seem to occur only in rheumatoid arthritis, or at least in joints where the chronic progression of the arthritis is clinically as well as pathologically indistinguishable from rheumatoid arthritis." Bennett † takes exception to this view, and Sherman[16] points to the occurrence of similar synovial changes, including lymphocytic nodules, in association with other types of chronic articular irritation. Such lesions include those caused by juxta-articular tumors or old fractures associated with severe degenerative joint disease. Nevertheless, there is always the possibility that in such instances rheumatoid arthritis coexisted with the other articular lesion, which may have acted as a predisposing factor. Thus, the controversy remains unresolved. It is evident, however, that lymphocytic nodules are at best a quantitative diagnostic criterion and that they are frequently absent, particularly in the early stages of the disease.

Formation of Fibrin or Fibrin-Like Deposits and Necrobiosis.—These changes appeared to be the result of an intense, localized form of tissue injury. Gross evidence of such changes was present in the form of pale yellow or white tags or deposits in five of the eight joints examined. Microscopically, fibrin-like material was found in every instance. As in the case of the proliferative process, these features tended to be most pronounced at the perichondrial margins, and they were often superimposed on the hypertrophied synovialis and pannus (Fig. 14). However, necrobiosis and fibrin-like deposits occurred in the absence of fixed-cell proliferation in the semilunar cartilages of Case 6 (Fig. 14). The high incidence of fibrin-like deposits and other localized changes in early or very active lesions was not stressed in the classic descriptions of rheumatoid arthritis based on long-standing lesions. Possibly this discrepancy reflects a difference in the intensity of the antecedent disease. Thus, it is of interest that the most pronounced deposition of fibrin-like material was present in Case 8, which had the severest constitutional reaction.

The nature of the fibrin-like material, or "fibrinoid," has long been the subject of controversy. Virchow ‡ first voiced the opinion that in acute serositis the intercellular components of the connective tissue beneath the surface exudate were altered to assume a fibrin-like appearance. Neumann[17] in 1880 coined the expression "fibrinoid degeneration" for this hypothetical process. However, he failed to state clearly how he differentiated this process from fibrinous inflammation and specifically included in his concept such structures as diphtheritic membranes, the linings of arteriosclerotic aneurysms, and tuberculous pleuritis, which are now generally considered to be largely fibrinous in origin. Since then, there have been repeated claims that "fibrinoid" could be distinguished from fibrin by various histologic or histochemical methods. However, in view of the questionable specificity of most of these methods and the variability in both the structural and the tinctorial properties of fibrin precipitates, particularly when admixed with other blood or tissue components, the evidence remains equivocal.[18] One of us § has been unable to demonstrate a consistent difference between fibrinous thrombi or serosal exudates, on the one hand, and the "fibrinoid" of rheumatoid nodules or articular lesions, on the other, by any of the following histologic techniques: periodic acid-Schiff reaction; toluidine blue after basic lead acetate fixation (for metachromasia[19]), photoelectric determination of dye-binding capacity (orange G and

† Bennett, G. A., in discussion on Collins.[15]

‡ Virchow, R., quoted by Neumann.[17]

§ Unpublished observations.

methylene blue) at controlled hydrogen ion concentrations,[20] fluorescent microscopy, Foot's reticulum stain, and Mallory's phosphotungstic acid hematoxylin stain.

Additional circumstantial evidence favoring a fibrinous origin of "fibrinoid" is found in the following observations: 1. In the "seven-day" lesion, the "fibrinoid" formed central patches overlying the superficial granulomas (Fig. 2), in at least some of which reticular fibrin emanated from certain inflamed vascular segments (Fig. 3). 2. Among the early lesions, there were all transitional forms between characteristic fibrinopurulent surface exudates (Fig. 11) and dense amorphous fibrin-like masses, which stained completely or only partially, if at all, with Mallory's phosphotungstic acid hematoxylin stain (Figs. 1, 13, and 18). 3. The fate of at least some of the "fibrinoid" appears to be either organization and replacement by dense hyaline fibrous tissue, as in fibrinous serositis,[21] or slough formation, as in the case of diphtheritic membranes. Why, under certain circumstances, fibrin fails to undergo resolution and becomes condensed into amorphous masses and why some such masses tend to resist organization are problems which merit further investigation.

The claim [6] that "fibrinoid" in articular lesions has diagnostic specificity was not substantiated. Identical-appearing material has been observed not only in other so-called connective tissue diseases, such as rheumatic fever and disseminated lupus erythematosus, but also in a variety of etiologically unrelated forms of arthritis, including tuberculous arthritis, staphylococcal arthritis, Charcot's joints, pigmented villonodular synovitis, and osteochondromatosis.

The relation between the formation of the fibrin-like material and the necrobiotic process is difficult to assess. In the two popliteal cysts, these reactions resembled those in rheumatoid nodules and showed a similar range of variation. Thus, in the 3½-month-old cyst, fragmentation of partially devitalized connective tissue was closely associated with permeation of fibrin-like material (Fig. 9), while the 7-month-old cyst showed a caseous type of necrosis and only occasional shreds of material that stained like fibrin with phosphotungstic acid hematoxylin (Fig. 15). In the joint lining proper, necrobiosis was less prominent and the fibrin-like material seemed more akin to that of fibrinous serositis. However, in the two earliest lesions, both reticular fibrinous and amorphous "fibrinoid" deposits were associated with focal granulomas, in which varying degrees of tissue disruption and cell damage were apparent (Fig. 5). In several lesions there were also regions where the superficial layer of palisaded synovial cells resembled the proliferative zone of rheumatoid nodules (Fig. 17), but no connection between this reaction and the fibrin-like deposits was evident. The occasional presence within the synovialis of typical rheumatoid nodules [22] or similar laminar foci of necrobiosis which lead to formation of fissures has been reported by others.[23] Such foci of necrobiosis or granulomatous inflammation could easily escape detection at gross examination and might conceivably result in sufficient irritation of the joint lining to set up either a fibrinous or a proliferative type of inflammation. There were also some indications that the fibrin-like material may be of more than secondary significance in the local devitalization of tissue. Thus, the cells enclosed in the fibrin-like material were nearly always sparse and often atrophic as compared with adjacent cells (Fig. 18). Moreover, the assumption that "fibrinoid" exerts a growth-inhibitory influence would serve to explain the resistance of the deposits to organization and their tendency to slough into the joint lumen. It is of interest that devitalization and impregnation with fibrin-like material involved the superficial cartilage in advance of pannus formation (Fig. 14).

Vascular Lesions.—The resemblance of the vascular lesions of "articular rheumatism" to those of dermatomyositis, periarteritis nodosa, syphilis, tuberculosis, malignant nephrosclerosis, etc., was recognized by Fahr [24] in 1921. Subsequently, Klinge [3] and more recently Christie [25] and Sokoloff and

associates [26] emphasized the systemic involvement of blood vessels in rheumatoid arthritis. Nevertheless, relatively little attention has been paid to the vascular changes in the articular process itself. In well-established lesions the changes are usually nonspecific in character, suggesting a secondary origin. However, a reconsideration of this problem appears indicated in view of the involvement of isolated minute vessels in early synovial lesions and the recently reported association of platelet and leucocytic thrombi with active rheumatic carditis.[27]

The commonest findings related to blood vessels were focal juxtavascular round cell infiltration and hyperplastic thickening of vessel walls (Fig. 16). Although such changes occur in almost any chronic inflammatory process, their pathogenesis and significance are not known. In some of the lesions, the capillary narrowing and concomitant intense fixed-cell proliferation, with increased tissue metabolism, may well have resulted in a relative ischemia (Fig. 11). The purplish gross appearance of the synovialis is presumably caused by the dilatation of the venous plexus.

Focal extravasation of red blood cells or hemosiderin deposits were present in at least 6 of the 10 biopsy specimens, possibly reflecting an increase in capillary "fragility." [28] In the tissue bordering on regions of necrobiosis, as in the walls of the popliteal cysts, capillary hemorrhage tended to be increased. Fibrin thrombi were also common in these regions (Fig. 15), but, as in the case of rheumatoid nodules,[22] it was difficult to decide whether such changes were primary or secondary. Regardless of the cause of thromboses, it is probable that the resulting ischemia was a contributory factor in the necrotizing process. That vascular obstruction is essential to necrobiosis may be questioned, since necrosis may occur in avascular cartilage (Fig. 14).

Active inflammation of isolated superficial capillaries (Fig. 3) and venules (Fig. 4) was present only in the "seven-day" biopsy specimen, and its acceptance as representative of rheumatoid arthritis must await confirmation. However, the absence of angiitis in other biopsy specimens does not exclude the possibility of its existence at an earlier stage in their development or elsewhere in the joint lining. The arteriolar thrombi in the "nine-day" lesion may well have been the remnants of such a process. Of the other lesions, only that of Case 3 was known to have been close to maximal clinical activity when examined. In this instance, the biopsy specimen consisted of fibrous parietal synovialis, which even in the "seven-day lesion," failed to show a distinctive angiitis.

Thus, the significance of the vascular involvement in the pathogenesis of rheumatoid arthritis remains a matter of speculation. Circulatory disorders secondary to the vascular involvement may result in further tissue damage, or the same causative agent may exert its injurious effect on both the vascular and the extravascular tissues. Whatever the mechanism, it would appear that primary changes in the circulatory system may well be important in determining the localization and character of the articular lesions in rheumatoid arthritis.

SUMMARY

The clinical and anatomic findings are described in eight cases of chronic symmetrical polyarthritis which fulfilled accepted clinical criteria required for the diagnosis of rheumatoid arthritis and in which knee biopsies were performed from seven days to nine months after the onset of symptoms or signs in the respective joints.

Marked variations from case to case and regional variations within the same joint made comparison of the tissue changes difficult. However, these relatively early and active articular lesions differed from the classic descriptions of the permanently deforming stage of rheumatoid arthritis in showing a closer resemblance to the type of tissue reaction characterizing the subcutaneous nodules and other systemic lesions of the disease.

A diffuse proliferative synovitis was a dominant feature in every instance. In the two earliest and mildest lesions (seven and

nine days' duration, respectively), this reaction was confined almost entirely to the intima. Typical lymphocytic nodules and villous hypertrophy were found only in three lesions, all of which were of more than four months' duration.

Evidence of more intense and localized injury was also a constant finding. Such injury was frequently superimposed on the diffuse proliferative process but varied independently in its degree of development and at times involved the fibrous joint capsule or articular cartilage. The involved regions were characterized by one or more of the following features, which correspond to those of rheumatoid nodules: (*a*) superficial or interstitial formation of fibrin or fibrin-like deposits; (*b*) necrobiosis; (*c*) minimal to moderate inflammatory cell reaction, usually mononuclear in type; (*d*) intense patchy edema and proliferation of large, primitive-appearing connective tissue cells, and (*e*) focal disruption of extracellular fibers and sequestration of thick, hyalinized collagenous bundles.

Focal or segmental vascular changes, involving particularly venules and capillaries, were associated in most instances with both the diffuse synovitis and the regions of intensified tissue injury. These changes consisted of inflammatory cell infiltration, hypertrophic thickening, with narrowing of the lumens, perivascular hemorrhages, or hemosiderin deposits, and, occasionally, fibrin permeation or thrombosis.

REFERENCES

1. Nichols, E. H., and Richardson, F. L.: Arthritis Deformans, J. Med. Res. **21**:149, 1909-1910.

2. Allison, N., and Ghormley, R. K.: Diagnosis in Joint Disease: A Clinical and Pathological Study of Arthritis, New York, William Wood & Company, 1931.

3. Klinge, F.: Die rheumatischen Erkrankungen der Knochen und Gelenke und der Rheumatismus, in Lubarsch, O., and Henke, F., Editors: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Springer-Verlag, 1934, Bd. IX/2.

4. Fisher, A. G. T.: Contribution to Pathology of the Rheumatoid Type of Arthritis and of Rheumatic Fever, Rheumat. Dis. **1**:46, 1939.

5. Steinberg, C. L.: Pathology of Atrophic Arthritis: Correlated Clinical and Laboratory Study, J. Lab. & Clin. Med. **27**:435, 1942.

6. Pique, J. A., and Schajowicz, F.: Importancia de la biopsia de la sinovial en las artritis reumatoideas o reumáticas, Rev. Asoc. méd. argent. **63**:381, 1949.

7. Collins, D. H.: Pathology of Articular and Spinal Diseases, Baltimore, Williams & Wilkins Company, 1950.

8. Parker, F., Jr., and Keefer, C. S.: Gross and Histologic Changes in the Knee Joint in Rheumatoid Arthritis, Arch. Path. **20**:507, 1935.

9. Hench, P. S., and Rosenberg, E. F.: Palindromic Rheumatism, a "New," Oft Recurring Disease of Joints (Arthritis, Periarthritis, Para-Arthritis) Apparently Producing no Articular Residues—Report of 34 Cases: Its Relation to "Angioneural Arthrosis," "Allergic Rheumatism" and "Rheumatoid Arthritis," Arch. Int. Med. **73**: 293, 1944.

10. Ropes, M. W., and Bauer, W.: Medical Progress: Rheumatoid Arthritis, Its Varied Clinical Manifestations, New England J. Med. **233**:618, 1945.

11. Rourke, M. D., and Ernstene, A. C.: Method for Correcting the Erythrocyte Sedimentation Rate for Variations in the Cell Volume Percentage of Blood, J. Clin. Invest. **8**:545, 1930.

12. Ropes, M. W., and Bauer, W.: Synovial Fluid Changes in Joint Disease, Cambridge, Harvard University Press, 1953.

13. Bennett, G. A., and Dällenbach, F. D.: Synovial Membrane Changes in Disseminated Lupus Erythematosus: Observations of 2 Autopsied Cases, Mil. Surgeon **109**:531, 1951.

14. Key, J. A.: Experimental Arthritis: Reactions of Joints to Mild Irritants, J. Bone & Joint Surg. **11**:705, 1929.

15. Collins, D. H.: Range of Pathologic Reactions Which Can Be Displayed by Human Synovial Tissues: Contribution to the Study of Specific Lesions, in Collins, D. H., Rheumatic Diseases, Philadelphia, W. B. Saunders Company, 1952.

16. Sherman, M. S.: Non-Specificity of Synovial Reactions, Bull. Hosp. Joint Dis. **12**:110, 1951.

17. Neumann, E.: Die Picrocarminfärbung und ihre Anwendung auf die Entzündungslehre, Arch. mikr. Anat. **25**:130, 1880.

18. Bennett, G. A.: Pathology of Connective Tissue, Fibrinoid Degeneration, in Ragan, C., Editor: Connective Tissues, Transactions of the First Conference, April 24-25, 1950, New York, Josiah Macy Jr., Foundation, 1951.

19. Wislocki, G. B.; Bunting, H., and Dempsey, E. W.: Metachromasia in Mammalian Tissues and Its Relationship to Mucopolysaccharides, Am. J. Anat. **81**:1, 1947.

20. Singer, M., and Wislocki, G. B.: Affinity of Syncytium, Fibrin, and Fibrinoid of the Human Placenta for Acid and Basic Dyes Under Controlled Conditions of Staining, Anat. Rec. **102**:175, 1948.

21. von Albertini, A.: Zum Begriff der fibrinoiden Degeneration, Schweiz. Ztschr. allg. Path. **6**:417, 1943.

22. Bennett, G. A.; Zeller, J. W., and Bauer, W: Subcutaneous Nodules of Rheumatoid Arthritis and Rheumatic Fever: Pathologic Study, Arch. Path. **30**:70, 1940.

23. Kersley, G. D., and Gibson, J. H.: Histopathology of Rheumatoid Arthritis, Especially in the Extra-Articular Manifestations, in Collins, D. H.: Rheumatic Diseases, Philadelphia, W. B. Saunders Company, 1952.

24. Fahr, T.: Zur Frage der Polymyositis (Dermatomyositis), Arch. Dermat. u. Syph. **130**:1, 1921.

25. Christie, G. S.: General Changes in Rheumatoid Arthritis, in King, E. S. J.; Lowe, T. E., and Cox, L. B., Editors: Studies in Pathology Presented to Peter MacCallum, Victoria, Melbourne University Press, 1950.

26. Sokoloff, L.; Wilens, S. L., and Bunim, J. J.: Arteritis of Striated Muscle in Rheumatoid Arthritis, Am. J. Path. **27**:157, 1951.

27. Stetson, C. A.: Pathogenesis of the Shwartzman and Arthus Phenomena and Their Relation to Human Rheumatic Fever, in Thomas, L., Editor: Rheumatic Fever: Symposium held at University of Minnesota, Nov. 29-Dec. 1, 1951, Minneapolis, University of Minnesota Press, 1952.

28. Robson, H. N., and Duthie, J. J. R.: Capillary Resistance and Adrenocortical Activity, Brit. M. J. **2**:971, 1950.

News and Comment

ANNOUNCEMENT

Program on Chemotherapy of Cancer.—Pathologists will be interested in the program for the wider exchange of information on chemotherapy of cancer which has been established by the Committee on Chemotherapy of the National Advisory Cancer Council in cooperation with the National Cancer Institute, the American Cancer Society, and the Damon Runyon Fund. All investigators interested in cancer chemotherapy are invited to take part in the program. Chairman of the Committee is Dr. Sidney Farber, director of the Children's Cancer Research Center, Boston.

A variety of activities will be developed throughout the program. Among them are (1) issuance of a periodic compilation of informal reports on current research in cancer chemotherapy; (2) formal conferences and symposiums on cancer chemotherapy; (3) compilation and issuance of a bibliography of cancer chemotherapy literature covering the period from 1946 through 1954, and (4) provision of opportunities for individuals and small groups to meet informally in various parts of the country for discussions of interinstitutional studies, standards for evaluating chemical agents, difficulties in this research area, potentially useful agents, and other topics the conferees consider pertinent.

In addition, the committee plans to bring to the attention of investigators chemical agents that may be of interest to them and to assist them in obtaining the chemicals in sufficient quantity for evaluation. Investigators who wish to take part in any of the activities of the program are invited to write to Dr. Gordon Seger, Secretary, Cancer Chemotherapy Committee, National Cancer Institute, Bethesda 14, Md.

Atlanta Graduate Medical Assembly—Southeastern Surgical Congress.—The Atlanta Graduate Medical Assembly and the Southeastern Surgical Congress will meet simultaneously in Atlanta, at the Biltmore Hotel, on Feb. 21-24. Among the pathologists on the program are Dr. William A. Meissner, of Boston, who will speak on "Diagnosis and Management of Thyroid Disease—A Pathologic Evaluation," and Dr. George Milles, of Chicago, who will speak on "Adenomatous Polyps of the Colon."

PERICARDIAL AND MYOCARDIAL VASCULARIZATION FOLLOWING CARDIOPERICARDIOPEXY

Magnesium Silicate Technique

AARON PLACHTA, M.D.; SAMUEL A. THOMPSON, M.D., and FRANCIS D. SPEER, M.D., New York

IN THE study of 10 autopsied patients following cardiopexy, the thoracic viscera, chiefly the hearts, were subjected to a technique for the visualization of newly formed and dilated preexisting pericardial, extracardiac, and intercoronary collateral anastomoses. The communicating extracardiac and intercoronary vascular channels were traced visually by the continuity of the histochemically stained endothelium. These blood vessels anastomosed with the traversing rami in the vascularized, granulomatous, adhesive pericarditis, serving as a bridge between the extracardiac and the intracardiac blood flow. The findings were further confirmed by serial sections of the injected hearts which were studied histologically, including the vessels of the granulomatous pericarditis, adjacent mediastinum, lungs, and diaphragm. In addition, roentgenographic radiopaque observations on the hearts were carried out in the conventional three-dimensional and the unrolled two-dimensional view. These studies suggest that the blood flow in these newly formed pericardial and dilated preexisting myocardial vessels may have been sufficient to sustain the functioning myocardium.

The present chain of events and the studies of many investigators of the surgical treatment of coronary heart disease were initiated by Heberden [12] through his description of the syndrome of angina pectoris. This clinical expression,[4] and the pathologic end-result of myocardial fibrosis and compensatory response of intercoronary anastomoses, have since become a matter of established fact. Surgical treatment for the anginal syndrome was proposed by François-Franck,[8] and the actual performance of sympathectomy was undertaken by Jonnesco [14] in a case of angina pectoris.

This set in motion investigations by Beck,* O'Shaughnessy,[20] and other modern surgeons,† who pointed toward the development of a practical surgical technique which would reduce the myocardial ischemia by enhanced collateral circulation.

Thompson's ‡ contribution through his animal experimentation with intrapericardial application of U. S. P. talc powder (hydrous magnesium silicate) yielded gratifying results. This procedure was applied to the human § with similar results, producing extracardiac and intracardiac coronary arterial anastomoses which seemed to establish adequate collateral circulation with dependable regularity.

CLINICAL DATA

For 14 years one of us (S. A. T.) has performed the operation upon a group of patients who were more or less completely incapacitated because of severe coronary heart disease. The rationale of the operation was to convert the ischemic myocardium into a relatively hyperemic myocardium. This was accomplished by distributing magnesium sili-

Dr. F. J. Borrelli and members of the Department of Radiology, and Dr. I. S. Kleiner and members of the Department of Biochemistry gave technical assistance.

From the Department of Pathology and Clinical Pathology (Dr. Plachta and Dr. Speer), and the Thoracic Surgical Service (Dr. Thompson), New York Medical College, Flower and Fifth Avenue Hospitals.

* References 2 and 3.

† References 11, 26, and 34.

‡ References 27 through 29.

§ References 30 through 32.

cate powder over the surfaces of the epicardium. The powder is extremely irritating and produces a foreign body reaction involving the surface of the pericardium, terminating in a vascularized, chronic, adhesive granulomatous pericarditis.

The operation is simple and requires no more than one-half hour for its completion. It consists in removing a portion of the fifth cartilage on the left side, where it is unnecessary to enter the pleura. The pericardial sac is opened for a length of approximately 1 to 2 in. (2.5 to 5 cm.). The surfaces of the heart are inspected and palpated for evidence of infarctions and adhesions. The pericardial fluid is aspirated as completely as possible, and from 4 to 6 gm. of dry, sterile magnesium silicate powder is widely distributed over the surfaces of the epicardium. The pericardial sac is loosely closed and the chest wall incision closed in anatomical layers without drainage.

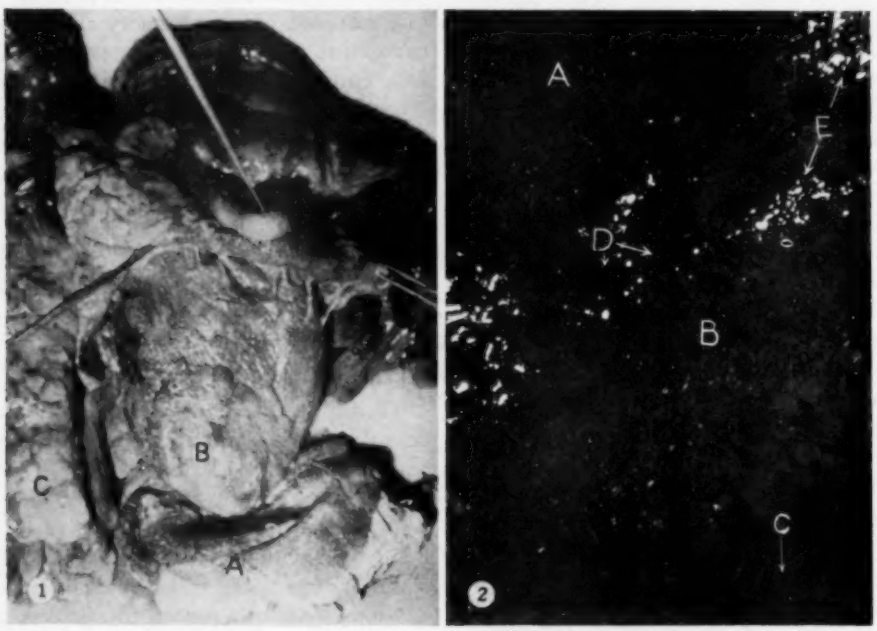


Fig. 1.—Stripped pericardium: (*A*) parietal; (*B*) visceral, showing diffuse, sticky, fibrinous, adhesive pericarditis ("bread-and-butter" type); (*C*) pleural and adjacent visceral hyperemia.

Fig. 2.—Polaroid study of Figure 1: (*A*) Edematous, hyperemic talc-induced pericarditis; (*D*) richly vascularized; (*B*) adherent, fibrinous, sticky, fused pericardium, showing lymphocytic, monocytic, eosinophilic, and plasma cell activity and giant cell formation; (*C*) preserved myocardial integrity, and (*E*) distributed talc granules; reduced ⅜ from mag. × 70.

Fifty-seven patients were operated upon from November, 1938, to July, 1951; a study was made of the cases to determine the degree of relief and improvement which had occurred and also to determine the length of life following the operation.

Seven of the patients died within three weeks after surgery. Of the 50 remaining patients, 5, or 10%, were improved less than 50% and were considered as having poor results; 25 patients were improved more than 50%, and 20 patients were improved more than 75%.

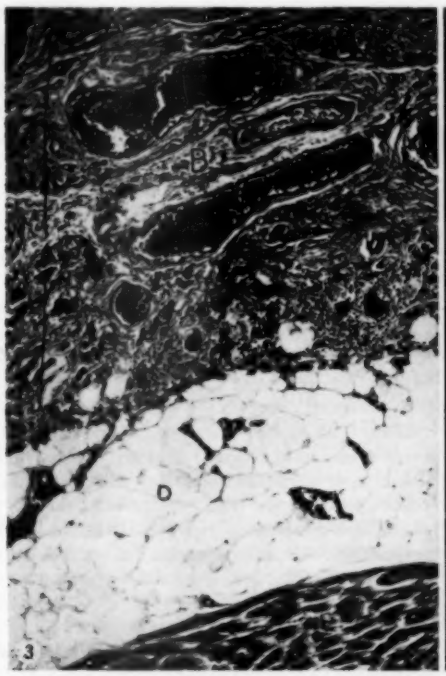
Thirty-three of the patients are still living, and the 17 who have died lived an average of five years after cardiopexy.

MATERIAL

The study which forms the basis of this report was initiated at the New York Medical College, Flower and Fifth Avenue Hospitals. There were two objectives: (1) to find a practical surgical

technique which would reduce myocardial ischemia by increased collateral pericardial circulation, and (2) to determine the source and the functional significance of such collateral circulation.

The preliminary experiments carried out on dogs and the subsequent reports on the functional significance of the established collateral circulation are a matter of record. The present report consists of human material collected over a period of 14 years. In the 1939-1951 period 17 patients on whom cardiopexy was performed have died. Ten patients of this group, consisting of both men and women, between 40 and 64 years of age, have been necropsied. The survival of these patients following cardiopexy was from 1 day to 10 years.



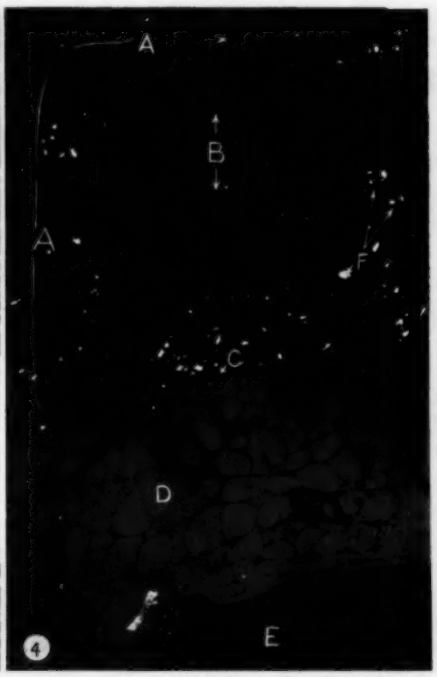


Fig. 3.—(*A*) Fibrous, adhesive granulomatous pericarditis, terminating (*C*) in the fused, eliminated visceral pericardium; (*B*) prominent vascular channels, round cell activity, phagocytosis, and giant cells; (*D*) hyperemic subepicardial adipose tissue, and (*E*) myocardium; reduced ⅓ from mag. × 80.

Fig. 4.—Polaroid study of Figure 3 showing (*F*) uniformly distributed talc crystals; reduced ⅓ from mag. × 80.

We shall confine our report of the autopsied cases chiefly to the heart and thoracic viscera, with special reference to the chronic pericarditis and the newly formed pericardial and preexisting vascular supply to the myocardium.

Case C101-40.—W. S., age 48. Necropsy, one day following cardiopexy, revealed severe, acute, diffuse serofibrinous pericarditis. This lesion, grossly and microscopically, was characterized by an acute aseptic ‖ inflammatory reaction, involving principally the pericardial membranes, demonstrated by severe hyperemia extending into the adjacent myocardium and extracardially to the neighboring organs—mediastinum, lungs, and diaphragm, associated with mediastinal, bronchial, and peribronchial lymphadenitis.

Case C102-39.—J. D., age 59. Necropsy, three days following cardiopexy, revealed an acute, diffuse, aseptic ‖ fibrinous pericarditis, involving the pericardial membranes, with absence of free fluid. The sticky fibrin film was arranged in irregular bands, simulating the "bread-and-butter" pericardium. The adjacent organs and tissues were severely hyperemic. The engorged, dilated vascular channels were grossly demonstrable in the pleurae, mediastinum, and surrounding tissues. In the heart the hyperemia extended into the endocardium. The fibrinous web in the pericarditis enclosed lympho-

‖ Aerobic and anaerobic cultures of pericardial membranes, adjacent organs, and blood from the left ventricle in the group under investigation were negative for growth after 144 hours' incubation.

cytes, monocytes, and many eosinophilic leucocytes. Early phagocytic activity, attempts toward giant cell formation, and plasma cell reaction were evident.

CASE C103-51.—H. B., age 60. Necropsy, nine days following cardiopexy, revealed a strikingly uniform fibrous, adhesive pericarditis. Vascular congestion was a common finding in the pericardium and adjacent organs, involving the mediastinum and pleura and extending into pulmonary parenchyma. Pericardial diffuse round cell infiltration, eosinophilic leucocytes, plasma cells, and multinucleated foreign-body giant cells were dominant (Figs. 1 and 2).

CASE C104-51.—R. W., age 45. Necropsy, 18 days following cardiopexy, revealed diffuse, uniform,

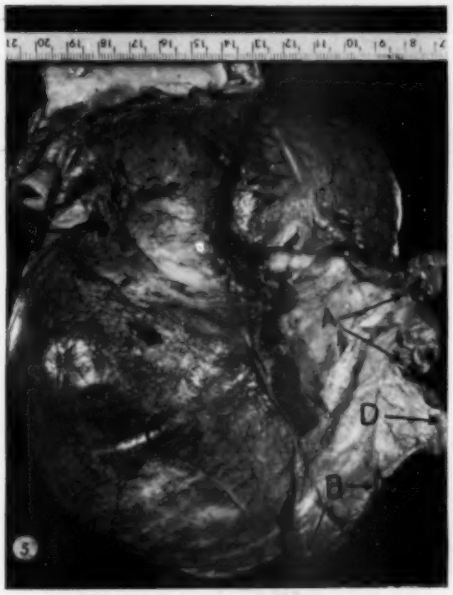


Fig. 5.—(*C*) Freely retractable, highly vascularized, adhesive granulomatous pericarditis, subjected to a technique for the visualization of newly formed and preexisting collateral anastomoses; (*A*) mediastinal, (*B*) diaphragmatic, and (*D*) pulmonary sources of collateral anastomoses.

fibrous, adhesive granulomatous pericarditis. This loosely arranged, freely movable fibrous connective tissue hugged the myocardium. The vascular channels were prominent, numerous, and compactly arranged. Engorgement was conspicuous. Fibroblastic proliferation eliminated the demarcations of the parietal and visceral pericardium. Lymphocytic and monocytic infiltration was common. Eosinophilic leucocytes, plasma cells, and multinucleated syncytial masses of cytoplasm with indefinite cell membranes were noted findings. Many of the giant cells simulated the Langhans' type, with ovoid nuclei radially arranged, several layers deep, at the periphery of the cell, in horseshoe fashion; cytoplasm contained phagocytosed material. Polaroid study revealed densely arranged talc crystals uniformly distributed throughout the proliferative fibrous tissues. Abundant pericardial newly formed vascular channels and dilated old vessels were a common encounter. Sections involving the adjacent mediastinum and pleurae revealed vascular collateral anastomoses extending to the adhesive granulomatous pericarditis, traversing the latter and terminating in intercoronary anastomoses.

CASE C105-40.—J. B., age 64. Autopsy, 21 days following cardiopexy, showed a diffuse, chronic, well-vascularized, adhesive granulomatous pericarditis. Patent congested lumina of newly formed and dilated preexisting blood vessels in the bridged pericardium freely anastomosed with the intercoronary system. The loosely adherent fibrous connective tissue was freely movable between the layers of the fused pericardium. Persistent leucocytic and histiocytic activity was conspicuous. Polaroid study revealed talc crystals uniformly distributed in the adhesive granulomatous reaction.

CASE C106-42.—J. H., age 63. Autopsy, 24 days following cardiopexy, gave findings similar to those noted in the preceding case.

CASE C107-51.—C. O., age 53. Necropsy, 92 days following cardiopexy, showed diffuse, chronic, fibrous, adhesive granulomatous pericarditis. Prominent vascularization of the pericardium was evident. The internal diameter of some of these vessels measured up to 0.10 cm. The granulomatous pericarditis contained vascular channels extending into the myocardium, anastomosing with branches of coronary arteries. The mediastinum, lungs, and diaphragm adhered to the granulomatous parietal pericarditis. These extrapericardial structures communicated with the myocardium through the vascularized, adherent pericardium. Pericardial eosinophilic, lymphocytic, and monocytic infiltration, associated with uniform distribution of talc crystals was an added constant finding (Figs. 3 and 4).

CASE C108-43.—S. N. E., aged 62. Necropsy, three and one-half years following cardiopexy, showed a diffuse, fibrous, adhesive granulomatous pericarditis. Prominent vascularization of the granulomatous pericardium, serving as a bridge for the collateral anastomoses between the extracardiac and the intracardiac blood flow, was striking. Mediastinal, pulmonary, and diaphragmatic adhesions were traversed by the pericardial collateral anastomoses, as traced by histochemically stained endothelium. Roentgenographic radiopaque study of pericardial injected vessels showed communication with intercoronary anastomoses in the myocardium. Large old, healed myocardial infarcts were vascularized. A movable, chronic granulomatous pericarditis was attached to the heart by delicate fibrous connective tissue, terminating at the fused

pericardium. Polaroid study showed the talc crystals to be uniformly distributed in the richly vascularized granulomatous pericardium (Figs. 5 through 11).

CASE C109-51.—C. H., age 40. Necropsy, seven and one-half years following cardiopexy, gave findings similar to those observed in the previously described cases, namely, persistent, uniform, diffuse, fibrous, adhesive, chronic granulomatous pericarditis, with abundant vascularization freely anastomosing with extracardiac vascular channels of the mediastinum, lungs, and diaphragm and with the intracardiac coronary system, extending to the endocardium (Fig. 12).

CASE C110-51.—B. O'H., aged 55. Necropsy, 10 years following cardiopexy, showed a diffuse, chronic, fibrous, adhesive granulomatous pericarditis. The mediastinum, lungs, and diaphragm were adherent to the anterior pericardium. The loosely adherent, freely retractable pericardium contained multiple congested and dilated vascular channels. These vessels intercommunicated with the coronary system, indicating abundant intracardiac anastomoses. Extracardiac collateral vessels leaving the mediastinum, lungs, and diaphragm extended through the newly formed and preexisting dilated channels in the granulomatous pericarditis and communicated with the coronary system. Highly vascularized, old, healed myocardial infarctions were conspicuous. Persistent leucocytic activity and uniformity of distribution of talc crystals were added features observed by Polaroid study (Figs. 13 through 15).

The cause of death of patients immediately following cardiopexy was found to be fresh or recent myocardial infarctions. In patients who survived 3 months, 3 years, 7 years, and 10 years after cardiopexy, the causes of death, respectively, were found to be perforation of aneurysm of the abdominal aorta, bilateral pneumonitis and sepsis, malignant hypertension with renal insufficiency, and acute myocardial insufficiency, with sudden death.

TECHNIQUE

We employed a technique [21] rendering the walls of the coronary artery system and the vessels of the pericardium, mediastinum, and diaphragm relatively impermeable by means of trinitrophenol-formalin-acetic acid endothelial fixation, preceded by washing with citrated and isotonic saline solutions. A rapid-staining method (trichrome stain: phenol, acid fuchsin, and ferric chloride) was used for the identification of endothelial continuity. The stain was combined with a suspension of potassium iodide in a powdered gelatin and agar. This radiopaque mixture was further combined with orange G or fast green FCF, scarlet R, and methylene blue, depending on the type of investigation (i. e., extracardiac or right or left coronary) of vascular

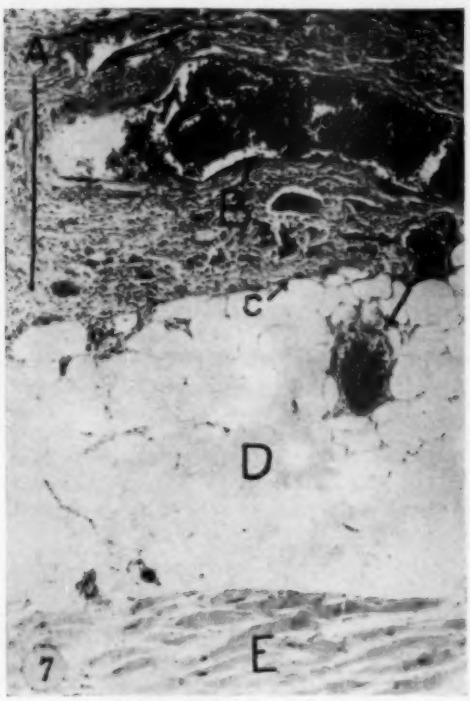
Fig. 6.—Unrolled view of radiopaque vascular outline of the pericardial perfused vessels in Figure 5, showing interanastomosis of (*C*) pericardial, (*A*) left coronary artery, circumflex, and anterior descending branches, and (*B*) right coronary artery and posterior descending branch.

continuity under consideration. This visual continuity of endothelium was carried out in combination with orange G or fast green FCF when extracardiac and pericardial collateral vascularization was being traced, and scarlet R and methylene blue were used, respectively, when the left and the right coronary artery system anastomosis was traced. The combined color scheme to aid visual dissection was carried out to ascertain the course, termination, and luminal diameter ratio of the extracardiac, pericardial, and intercoronary anastomoses. The method was a one-step procedure, permitting both roentgenographic cardiac vascular study in the conventional three-dimensional and the unrolled two-dimensional view and visual dissection for the observation of endothelial continuity. Radiopaque studies ¶ were carried out to satisfy the criteria imposed by other observers for conclusive evidence of vascular intercommunication.

Specific studies, as referred to above, were carried out on four patients, who survived over 3 months, 3 years, 7 years, and 10 years. The four hearts referred to correspond to Necropsies C107-51, C108-43, C109-51, and C110-51. Two of the hearts referred to (C109-51 and C110-51), with combined extracardiac vascular study, were subjected to the above method in its entirety, and two hearts (C107-51 and C108-43) were subjected only to pericardial and coronary vascular perfusion, to ascertain the intercommunication with the intercoronary system. In all instances the right and left coronary arteries were perfused by the respective radiopaque color schemes. When the right coronary artery was injected with the basic methylene blue dye, the material was recovered in the left coronary sinus

¶ We believe that the roentgenogram silhouettes showing the radiopaque vascular outlines are deceptive in giving the appearance of coronary intercommunications. In reality the overlapping vessels bear no relation to true interanastomoses. Roentgenograms should be used as a guide to facilitate the evaluation, not as a diagnostic means.

and revealed in its tributaries. A similar result was obtained when the left coronary artery was perfused with scarlet R. Likewise, when a pericardial vessel was perfused with orange G or fast green FCF, intercoronary vascular channels, as well as the coronary sinus, contained the corresponding dye, indicating pericardial, extracardiac, and intracardiac anastomosis. Serial sections of the injected hearts, between 250 to 500 μ, were made and studied histologically to reveal the vessels of the granulomatous pericarditis, adjacent mediastinum, lungs, and diaphragm. The microscope screw-micrometer eyepiece (Spencer) was used for the vascular micron-



Legend on facing page.

diameter evaluation. By this calculation the inner diameter of some of the blood vessels in the revascularized granulomatous pericarditis measured up to 650 μ.

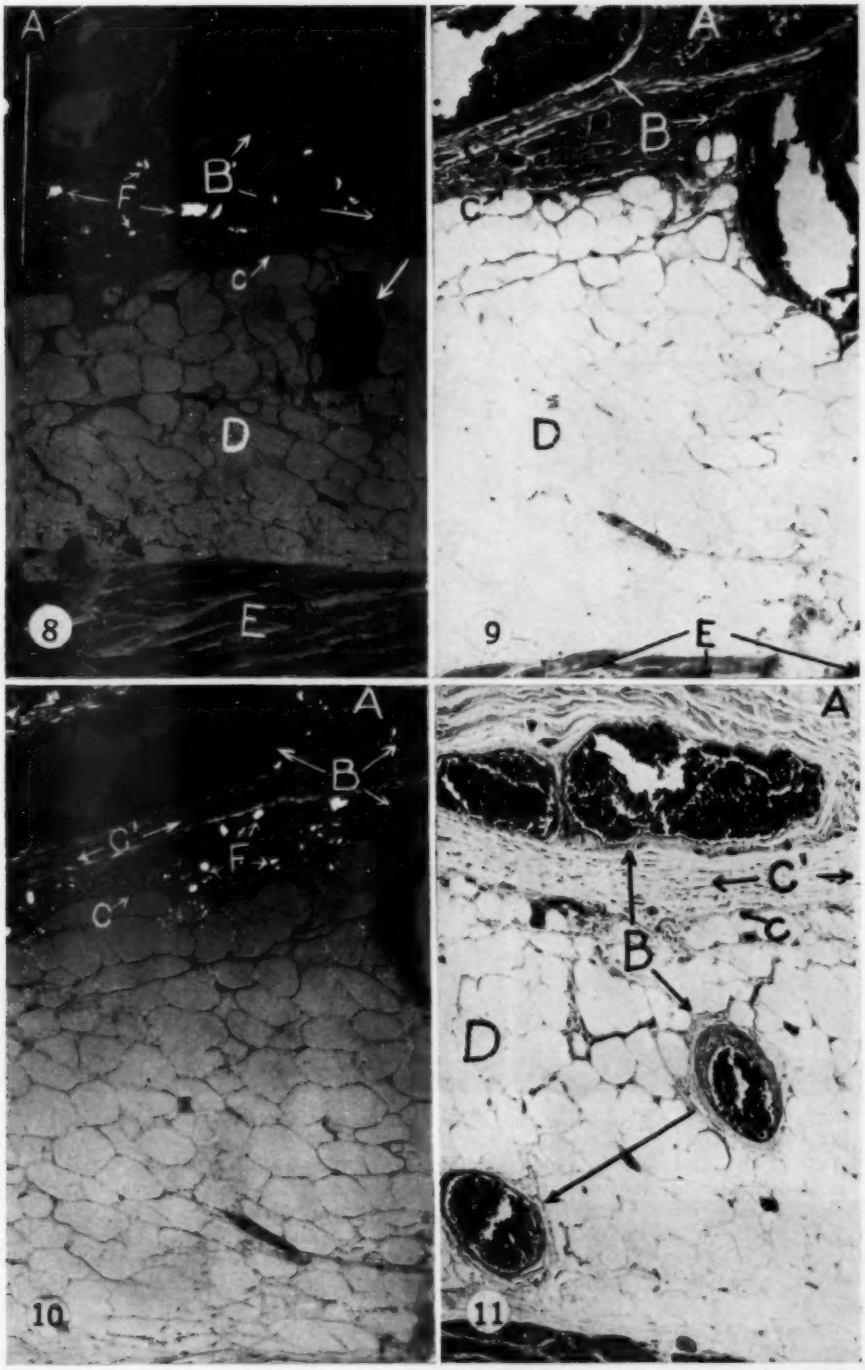
The dissection further disclosed the extent of communication between the extracardiac and the intracardiac anastomoses, through the traversing rami housed in the granulomatous pericarditis. Microscopic study of serial sections confirmed the existence of these anastomoses by undisturbed continuity of stained endothelium.

RÉSUMÉ AND EVALUATION OF FINDINGS

The gross and microscopic findings in these hearts revealed a uniform, acute, aseptic inflammatory lesion of the parietal and visceral pericardium. Degeneration and disappearance of the mesothelium lining the surfaces of the pericardial membranes and subsequent fusion of the latter by proliferative fibroblastic activity were noted. The inflammatory pericardium was characterized by severe hyperemia extending through the myocardium to the endocardium and extracardially to the adjacent organs. Diffuse infiltration of lymphocytes and monocytes was strikingly confined to the fused pericardial membranes. The maximum inflammatory activity was reached on the third day after the introduction of hydrous magnesium silicate. After the peak of inflammatory activity a notable transition took place, characterized by the appearance of eosinophilic leucocytes and plasma cells and the deposition of a sticky fibrin. Early phagocytic activity and a dry plastic pericarditis were outstanding and dominant features. Gradual development of multinucleated foreign body giant cells in a loose connective tissue stroma was apparent. Free communications between extracardiac collateral channels and intracardiac channels through the newly formed and preexisting dilated vessels in the bridged granulomatous pericarditis were present in abundant quantities 18 days after surgery. This supported the contention of other investigators # that free cardiac-extracardiac communications in the adhesive pericarditis were present 14 days after surgery. The loosely adherent, vascularized granulomatous pericarditis permitted free cardiac mobility. The vessels in the granulomatous pericarditis interbridging the extracardiac and intracardiac circulations measured up to 650 μ in diameter. The striking features of the lesion when fully developed were its persistence, uniformity, and stability, which did not deviate with a lapse of many years following surgery.

The important physiological significance was revealed by the extensive anastomoses between the cardiac vessels and the vessels of the neighboring organs and other structures through the granulomatous pericarditis.

References 7, 17, and 19.



Figs. 7, 9, and 11.—Left ventricle, right ventricle, and apex, respectively: (*A*) granulomatous; (*B*) highly vascularized, adhesive pericarditis, showing active leucocytosis, plasma cells, and giant cells; (*C*) fused, eliminated visceral pericardium; (*C'*) delicate fibrous junctional layer acting as a buffer in preserving the integrity of (*D*) subepicardium and (*E*) myocardium; × 70.

Figs. 8 and 10.—Polaroid study of Figures 7 and 9, showing (*F*) uniformity and persistence of talc crystals; × 70.

The extracardiac vascular collateral circulation formed in several regions and was constant. Among the common sources of the vascular plexuses were the mediastinum, the pleurae, the roots of the lungs, the diaphragm, and ramifications of the coronary arteries and cardiac veins. This common cardiopericardial vascularization was found greatly augmented in the talc-induced adhesive pericarditis. The most extensive anastomotic network was noted in the fused granulomatous pericardium, subepicardium, myocardium, and endocardium. The venous plexus was directed into the heart chambers through the coronary sinus and foramina venarum minimarum, communicated with the azygos system and the internal mammary veins through their radicles adjacent to the pericardium, found ostia at the terminations of the superior and inferior venae cavae, and anastomosed openly with the pulmonary venous system at the cardiac impressions. Observed sources of arterial blood, beside the coronary arteries, were the vasa vasorum of the pulmonary arteries and of the root of the aorta; ramifications of the pulmonary arteries in the parenchyma of the lungs near the cardiac impressions; the intercostal arteries near their origin; the bronchial arteries; pericardiac and esophageal branches of the tho-

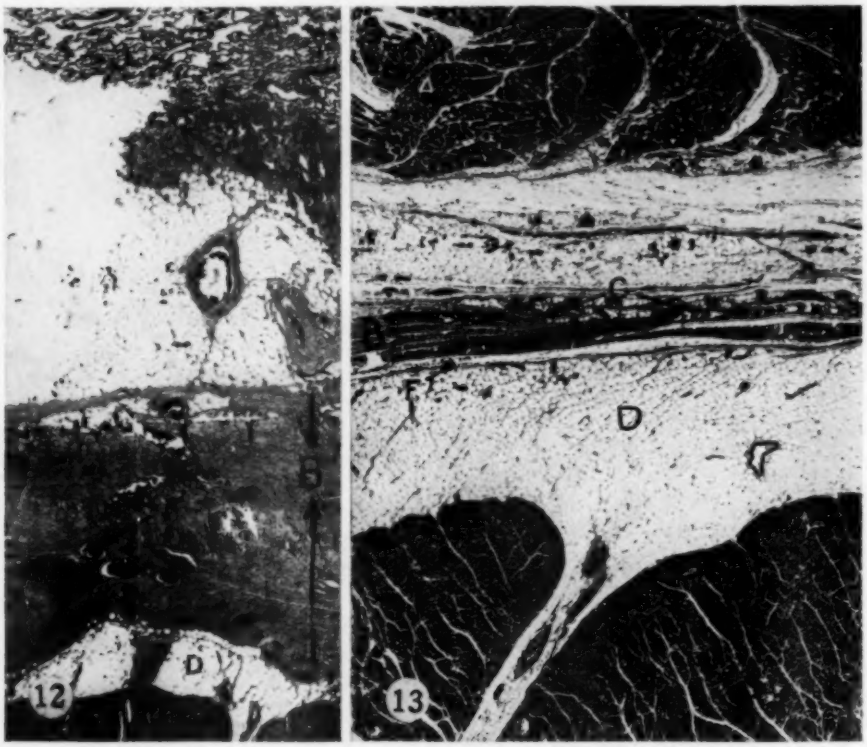


Fig. 12.—Extracardiac source: pulmonary, mediastinal, pericardial, and intercoronary collateral anastomoses: (*A*) oxygenated blood from lungs passing through (*F'*) vessels in pleura by way of (*B*) granulomatous, highly vascularized pericarditis; (*F*) large vessels leaving highly vascularized granulomatous pericarditis, traversing preserved (*D*) subepicardium, anastomosing in preserved (*E*) myocardium; × 10.

Fig. 13.—(*A*) Diaphragmatic extracardiac source of collateral circulation through the (*C*) highly vascularized, (*B*) granulomatous pericarditis, showing (*F*) fibrous connective tissue junctional layer serving as a buffer, preserving the integrity of (*D*) subepicardium and myocardium; × 10.

racic aorta; the superior phrenic arteries; the pericardiophrenic arteries, and the anterior mediastinal, thymic, pericardiac, and sternal branches of the internal mammary arteries.

COMMENT AND SUMMARY

Merkel [18] supported Thorel's [23] suggestion that in the event of obliteration of both coronary arteries the heart might be supplied with blood through the vessels in formed pericardial adhesions. O'Shaughnessy and associates [20] observed that when pericardial adhesions were produced between the heart and the mediastinum, vascular connections occurred. The intercommunications appeared two weeks after the operations. Moritz and associates [19] demonstrated pericardial vascularization in rheumatic adhesive pericarditis.

Our own investigations disclosed the nature, development, and behavior of the talc-produced granulomatous pericarditis and its vascularization following cardiopexy. The supposition that the granulomatous pericarditis becomes a static, avascular, healed scar is without basis. We believe that the U. S. P. talc powder (hydrous magnesium silicate) serves as a trigger device, irritating the pericardial membranes and initiating vasodilatation, which terminates in pericardial adhesions and collateral vascularization. This function by the ever-present talc crystals is evidently a ceaseless activity. The lesion is constantly characterized by hyperemia and, presumably, by accelerated flow of blood through newly formed and preexisting dilated vascular channels in the chronic, adhesive granulomatous pericarditis, which we know persists for at least 10 years after operation. We believe such a lesion will actively persist as long as the patient lives.

The clinical features of constrictive pericarditis, such as prominent neck veins, hepatomegaly and ascites, hypotension and an increase in venous pressure, and fluoroscopically fixed heart were not seen in any of our cases. On the contrary, the heart in all our cases retained its integrity both anatomically and physiologically.

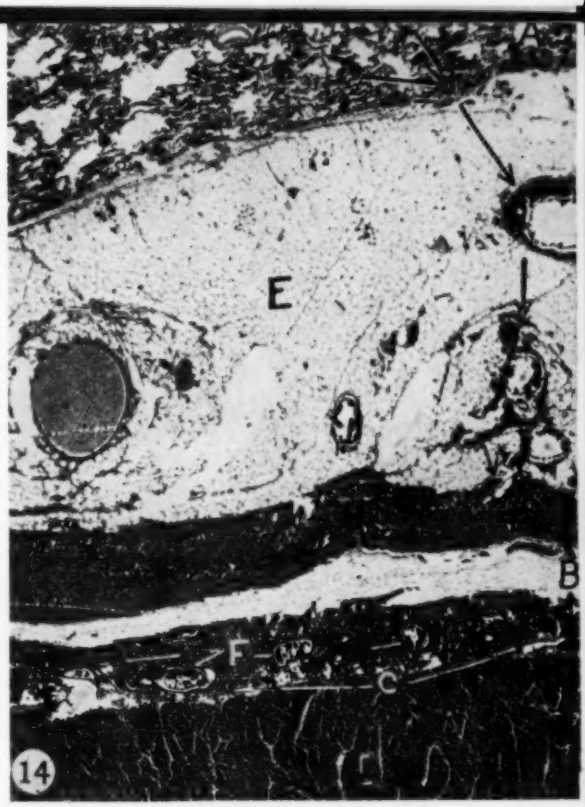


Fig. 14.—(*A*) Pulmonary, (*E*) bridging mediastinal, extracardiac collateral circulation anastomosing through (*B*) adhesive granulomatous, (*F*) highly vascularized bridged pericarditis; (*C*) junctional connective tissue fibrous layer, and preserved myocardium; × 10.

Black recently reported * that the epicardium formed a barrier to the ingrowth of blood vessels and was the reason for the past failures in extracardiac revascularization of the myocardium. To overcome this barrier, he and his associates practiced deepicardiali-

* Black, H., in discussion on Bailey,[1] p. 171.

Fig. 15.—Polaroid study of (*A*) granulomatous pericarditis, containing uniformly distributed (*F*) talc crystals, in a case 10 years after cardiopexy; reduced 2/5 from mag. × 10.

zation by the local application of 92% phenol. It seems questionable whether such a procedure is necessary—and whether it may not ultimately result in a constricting fibrosis.

Talc-induced pericarditis gradually but completely eliminates the barrier, as evidenced by the absence of mesothelium and of the supportive thin layer of connective and elastic tissue fibers of both parietal and visceral pericardial membranes. The initiated fibroblastic proliferation, with subsequent fusion of these membranes, results in a progressive, chronic, adhesive, vascularized, granulomatous pericarditis.

The usefulness of the rami telae adiposea † in providing anastomoses between the coronary arteries and the vessels of the surrounding structures cannot be overestimated. The greatest importance may be attached to the increase in their numbers, far offsetting the so-called "critical micron diameter" advocated by Schlesinger.[28] He and his associates [28] documented the so-called critical 40 μ pathway functionally necessary to prevent the myocardial effects of the occlusion of a large coronary artery. This conclusion was derived from studies on collateral coronary circulation utilizing a modified method of Gross, in which radiopaque lead acetate-agar suspension was used for roentgenographic evaluation.

Prinzmetal and associates,‡ utilizing Dock's [6] perfusion method, provided evidence that a 10 μ diameter vessel will accommodate passage of a radiopaque mixture having physical properties similar to blood. Such vessels, if sufficient in number, may sustain myocardial function. This evidence was further supported by Goldman and associates.[9]

The hearts in our series, studied by means of histochemical endothelial staining, visual dissection, and roentgenographic and microscopic evaluation, showed the course and origin of the collateral vessels to correspond to those reported by previous investigators. In accord with observations by Gross,[10] Hudson,[13] and Lezius,[16] we found the coronary system anastomosing through the vascularized granulomatous pericarditis with vessels in the mediastinum, parietal pericardium, diaphragm, and hiluses of the lung, vasa vasorum of the ascending aorta, and pericardial branches of the internal mammary arteries.

The extracardiac anastomoses, we believe, constitute a most significant reserve for cardiac circulation, augmented by the presence of vascularized pericardial adhesions. The clinical evidence referable to talc pericarditis and previously reported lends reasonable support to the adequacy of this newly formed and stimulated preexisting pericardial collateral circulation.

† References 5 and 15.

‡ References 22 and 23.

REFERENCES

1. Bailey, C. P.; Truex, R. C.; Angulo, A. W.; Gleckeler, G. D.; Likoff, W.; Antonius, N., and Neptune, W. B.: Anatomic (Histologic) Basis and Efficient Clinical Surgical Technique for the Restoration of the Coronary Circulation, J. Thoracic Surg. **25**:143, 1953.

2. Beck, C. S.: Development of a New Blood Supply to the Heart by Operation, Ann. Surg. **102**:801, 1935.

3. Beck, C. S.: Revascularization of the Heart, Surgery **26**:82, 1949.

4. Blumgart, H. L.; Zoll, P. M., and Wessler, S.: Angina Pectoris: Clinical Pathologic Study of 177 Cases, Tr. A. Am. Physicians **63**:262, 1950.

5. Bohning, A.; Jochim, K., and Katz, L. N.: Thebesian Vessels as Source of Nourishment for Myocardium, Am. J. Physiol. **106**:183, 1933.

6. Dock, W.: Capacity of the Coronary Bed in Cardiac Hypertrophy, J. Exper. Med. **74**:177, 1941.

7. Eckstein, R. W.; Gregg, D. E., and Pritchard, W. H.: Magnitude and Time of Development of the Collateral Circulation in Occluded Femoral Carotid and Coronary Arteries, Am. J. Physiol. **132**:351, 1941.

8. François-Franck: Signification physiologique de la résection du sympathique dans la maladie de Basedow, l'épilepsie, l'idiotie et le glaucoma, Bull. Acad. Méd. **41**:565, 1899.

9. Goldman, A.; Shaw, C.; Corday, E.; Fields, J.; Kennamer, S. R.; Smith, A.; Lindgren, I., and Prinzmetal, M.: Experimental Methods for Detection of Changes of Blood Supply to the Heart, J. Thoracic Surg. **24**:105, 1952.

10. Gross, L.: Blood Supply to the Heart in Its Anatomical and Clinical Aspects, New York, Paul B. Hoeber, Inc., 1921, p. 171.

11. Gross, L.; Blum, L., and Silverman, G.: Experimental Attempts to Increase the Blood Supply to the Dog's Heart by Access of Coronary Sinus Occlusion, J. Exper. Med. **65**:91, 1937.

12. Heberden, W.: Some Account of a Disorder of the Breast, M. Tr. Coll. Physicians **2**:59, 1786.

13. Hudson, C. L.; Moritz, A. R., and Wearn, J. T.: Extracardiac Anastomoses of the Coronary Arteries, J. Exper. Med. **56**:919, 1932.

14. Jonnesco, T.: Angine de poitrine guérie par la résection du sympathique cervico-thoracique, Bull. Acad. méd. **84**:93, 1920.

15. Lendrum, B.; Kondo, B., and Katz, L. N.: Role of Thebesian Drainage in the Dynamics of Coronary Flow, Am. J. Physiol. **143**:243, 1945.

16. Lezius, A.: Die künstliche Blutversorgung des Herzmuskels, Arch. klin. Chir. **189**:342, 1937.

17. Mendlowitz, M.; Shauer, G., and Gross, L.: Hemodynamic Studies in Experimental Coronary Occlusion: II. Closed Chest Experiments, Am. Heart J. **13**:664, 1937.

18. Merkel, H.: Zur Kenntnis der Kranzarterien des menschlichen Herzens, Verhandl. deutsch. path. Gesellsch. **10**:127, 1906.

19. Moritz, A. R.; Hudson, C. L., and Orgain, E. S.: Augmentation of the Extracardiac Anastomoses of the Coronary Arteries Through Pericardial Adhesions, J. Exper. Med. **56**:927, 1932.

20. O'Shaughnessy, L.; Slomb, D., and Watson, F.: Surgical Revascularisation of the Heart: Experimental Basis, Lancet **1**:617, 1939.

21. Plachta, A., and Speer, F. D.: Method for Visual and Roentgenogram Tracing of Extracardiac and Intracardiac Vascular Continuity, to be published.

22. Prinzmetal, M.; Simkin, B.; Bergman, H. C., and Kruger, H. E.: Studies on the Coronary Circulation: II. Collateral Circulation of the Normal Human Heart by Coronary Perfusion with Radioactive Erythrocytes and Glass Spheres, Am. Heart J. **33**:420, 1947.

23. Prinzmetal, M.; Schwartz, L. L.; Corday, E.; Spritzler, R.; Bergman, H. C., and Kruger, H. E.: Studies on the Coronary Circulation: VI. Loss of Myocardial Contractility After Coronary Artery Occlusion, Ann. Int. Med. **31**:429, 1949.

24. Raney, R. B.: Hitherto Undescribed Surgical Procedure Relieving Attacks of Angina Pectoris: Anatomic and Physiologic Basis, J. A. M. A. **113**: 1619, 1939.

25. Schlesinger, M. J.: Injection Plus Dissection Study of Coronary Artery Occlusion and Anastomoses, Am. Heart J. **15**:528, 1938.

26. Stephens, H. B., and Benteen, H.: Muscle Grafts in the Surgery of Heart and Lungs, California & West. Med. **49**:366, 1938.

27. Thompson, S. A.: Development of Cardio-Pericardial Adhesions Following the Use of Talc, Proc. Soc. Exper. Biol. & Med. **40**:260, 1939.

28. Thompson, S. A.: Operation for the Relief of Coronary Artery Disease: Preliminary Report, Quart. Bull. Sea View Hosp. **5**:175, 1940.

29. Thompson, S. A., and Raisbeck, M. J.: Cardio-Pericardiopexy: Surgical Treatment of Coronary Arterial Disease by the Establishment of Adhesive Pericarditis, Ann. Int. Med. **16**:495, 1942.

30. Thompson, S. A., and Raisbeck, M. J.: Surgical Rehabilitation of the Coronary Cripple, Ann. Int. Med. **31**:1010, 1949.

31. Thompson, S. A., and Plachta, A.: Fourteen Years' Experience with Cardiopexy in Treatment of Coronary Artery Disease, J. Thoracic Surg. **27**:64, 1954.

32. Thompson, S. A., and Plachta, A.: Experiences with Cardio-Pericardiopexy in the Treatment of Coronary Disease, J. A. M. A. **152**:678, 1953.

33. Thorel, C.: Pathologie der Kreislauforgane: Pathologie der Koronargefässe, in Lubarsch, O., and Ostertag, R.: Ergebnisse der allgemeinen Pathologie und pathologischen Anatomie des Menschen und der Tiere, Wiesbaden, J. F. Bergmann, 1903, Vol. 9, p. 559.

34. von Wedel, J.; Stone, P. W.; Neumann, C. G.; Lord, J. W.; Hinton, J. W., and Moran, R. E.: Revascularization of the Heart by a Pedicle Skin Flap, Science **116**:319, 1952.

35. Zoll, P. M.; Wessler, S., and Schlesinger, M. J.: Interarterial Coronary Anastomoses in the Human Heart, with Particular Reference to Anemia and Relative Cardiac Anoxia, Circulation **4**:797, 1951.

POLYCYSTIC LIVER

Analysis of Seventy Cases

P. J. MELNICK, M.D., Eloise, Mich.

POLYCYSTIC liver is comparatively rare. In 1906 Moschcowitz [1] collected 85 cases from the literature, added 6 cases, and outlined the three main theories of origin of this disease. Cruveilhier and Virchow had believed that polycystic liver resulted from cystic dilatation of bile ducts following inflammatory strictures. Later von Hippel thought it was neoplastic. Toward the end of the 19th century Borst, Borrmann, and others believed the condition to be a form of embryonal maldevelopment. Moschcowitz believed that this maldevelopment consisted of the presence of aberrant bile ducts which underwent cystic dilatation.

Lewis [2] in 1912 described the embryologic basis for an understanding of these aberrant bile ducts. The distal portion of the liver anlage forms the hepatic parenchyma, and the proximal portion forms the duct system, which then grows into the rapidly enlarging distal hepatic portion. The terminal branches of the duct system reach to the liver lobules and form the interlobular bile ducts in the portal septa. In the meantime, small bile ducts begin to appear in the liver parenchyma, the so-called intralobular or intrahepatic bile ducts, which connect the bile canaliculi to the interlobular bile ducts in the portal septa. They can be seen in 10 mm. embryos and are abundant in embryos more than 25 mm. in length. In neonatal life they are represented by the canals of Hering. Lewis concluded that these intrahepatic bile ducts differentiate from the liver cell cords.

Meyenburg [3] in 1918 described the occurrence in polycystic liver of groups or clusters of small bile ducts in the liver lobules separate from the portal areas; these have since been known as Meyenburg's complexes. He interpreted these as persisting intrahepatic bile ducts. Many more intrahepatic bile ducts are formed in the embryo than are necessary, and normally there is involution of the excess ducts. Meyenburg's complexes are groups of these intrahepatic bile ducts that fail to involute, and polycystic liver results from their gradual cystic dilatation (Figs. 1 and 2). This concept of the origin of polycystic liver has been supported by many authors; reviews of the literature by Kaufmann,[4] Hanser,[5] and Norris and Tyson [6] emphasize this view.

The present study was motivated by the possibility of learning more about the origin of the intrahepatic bile ducts from a study of polycystic liver. Lewis' concept of their hepatocellular origin is supported by the work of Bloom,[7] who demonstrated these ducts even in 1 mm. embryos, and also by recent studies on the Carnegie collection of embryos by Streeter.[8] Bloom reviewed the literature and pointed out that Hertwig and other embryologists agreed with this view, but that Minot and others believed that they are formed from the proximal, or bile-duct-forming, segment of the hepatic anlage. Some embryologists in both groups make no distinction between the interlobular and the intrahepatic bile ducts. Still others, such as Hammar,[9] agree with Lewis that intrahepatic bile ducts differentiate from the liver cells

Read before the Section on Pathology and Physiology at the 103d Annual Meeting of the American Medical Association, San Francisco, June 23, 1954.

From the Department of Pathology, University of Southern California School of Medicine, and the Laboratory of the Los Angeles County General Hospital. Present address: Wayne County General Hospital, Eloise, Mich., and Wayne University College of Medicine, Detroit.

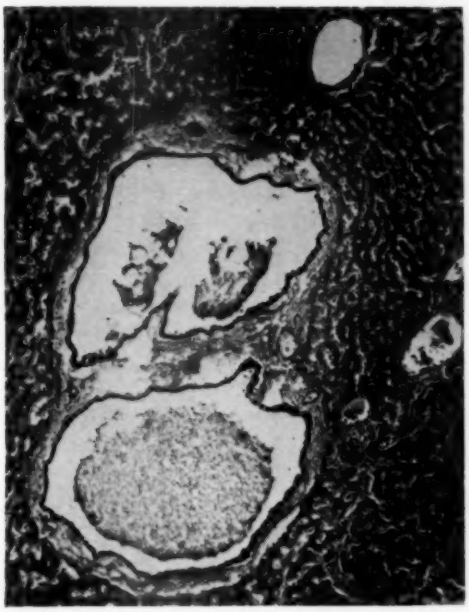


Fig. 1.—Example of cysts in polycystic liver. The cyst lining takes the stain in the same manner as do the bile capillaries (the latter are not visible at this magnification). Eppinger bile capillary stain; reduced ¼ from mag. × 100.

but believe that the proximal bile-duct-forming segment arises from a second, separate, entodermal anlage. These different views have had varying influences on the interpretation not only of the pathogenesis of polycystic liver (Wackerle,[10] Hanser [5]) but also of the origin of bile duct proliferation in portal cirrhosis of the liver, and of regenerative processes of the liver in general (Herxheimer and Thölldte [11]).

MacMahon and Thannhauser [12] and MacMahon and other associates have reported studies of the liver from several children with xanthomatosis and jaundice; in these livers there is complete congenital absence of the interlobular bile ducts in the portal septa, yet the canals of Hering are clearly seen and are increased. The canals of Hering represent the intrahepatic bile ducts in neonatal life and must have been formed from the liver cell cords, since there are no interlobular bile ducts present.

Bile duct formation can also be seen in many cases of hepatocellular carcinoma; Edmondson and Steiner [13] and Berman [14] have described the polymorphism of this tumor and the formation of bile duct elements from neoplastic liver tissue.

Blum and Müller [15] in 1932 described, in a polycystic liver from an 83-year-old man, the unique occurrence of new-formation of intrahepatic bile ducts from liver cell cords, such as normally occurs only in the embryo. Among the 70 cases of polycystic liver reported in this study there were encountered 2 cases similar to the 1 reported by Blum and Müller. In the livers of both of these cases there are seen different stages of formation of intrahepatic bile ducts directly from the liver cells; the newly formed bile ducts are seen in various stages of cystic dilatation. The process presents an organoid pattern and retains a regular arrangement, although it appears to stand at the borderline of neoplasia. These two rare cases are described in detail below.

Fig. 2.—Example of Meyenburg's complex in polycystic liver. The lining epithelium of the bile duct elements forming the complex generally takes the stain; the epithelium of the interlobular bile duct in the adjacent portal septum does not take the stain. Eppinger bile capillary stain; reduced ¼ from mag. × 150.

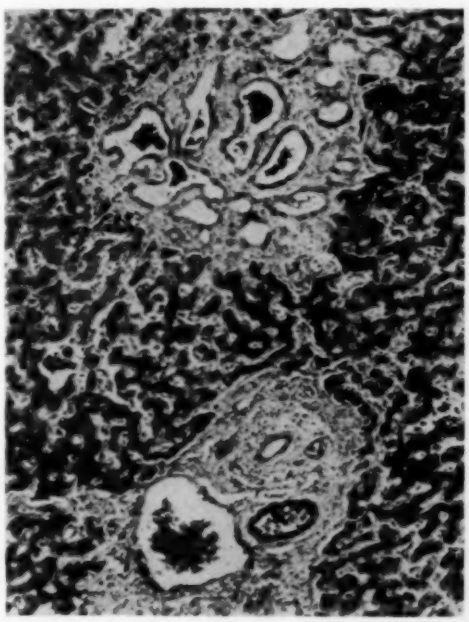


TABLE 1.—*Classification by Age of Seventy Cases of Polycystic Liver*

Age by Decades	Cases, No.
0 to 10	2
11 to 20	0
21 to 30	1
31 to 40	5
41 to 50	13
51 to 60	11
61 to 70	14
71 to 80	15
81 to 90	7
91 to 100	2

TABLE 2.—*Incidence of Polycystic Liver According to Sex*

Sex	Cases, No.
Male	36
Female	34

ANALYSIS OF SEVENTY CASES

In the autopsy records of the Los Angeles County General Hospital a total of 70 cases of polycystic liver is recorded over a 36-year period, an incidence of 1 per 687 autopsies. Davis [16] collected 499 cases from the literature up to 1937. The largest single series that can be found in the recent literature is a study of 24 cases by Comfort and Gray [17] in 1952. The accompanying tables are summaries of the significant data abstracted from the clinical and autopsy records of the 70 cases.

TABLE 3.—*Incidence of Polycystic Liver According to Race or Nationality*

	Cases, No.
Caucasian	57
Negro	11
Mexican	2

TABLE 4.—*Classification of Cases According to Weight of Polycystic Livers*

Weight of Polycystic Livers, Gm.	Cases, No.
Less than 1,000	2
1,000-1,500	27
1,500-2,000	31
2,000-2,500	8
2,800	1
4,400	1

It is evident that polycystic liver develops progressively over the years (Table 1) and is compatible with the gradual cystic dilatation of Meyenburg's complexes. The condition is rarely seen in children.

The largest liver was extensively involved by cysts (Table 4). Such extensive involvement is rare. In spite of marked reduction in the amount of liver parenchyma, no evidence of impaired liver function has been observed in these cases.

TABLE 5.—*Classification of Cases According to Number of Cysts*

Cysts, No.	Cases, No.
Few	8
Moderate number	19
Many	43

TABLE 6.—*Classification of Cases by Size of Cysts* *

Largest Diameter of Cysts, Cm.	Cases, No.
1	10
2	19
3	12
4	4
5	8
6	3
7	3
8	3
9	1
10	5
11	..
12	2

* The larger cysts were generally seen in the older age groups.

TABLE 7.—*Incidence of Cases According to Types of Epithelium Lining the Cysts* *

Epithelium Lining the Cysts	Cases, No.
Cuboidal	48
Low cuboidal	16
Flat	4
No	2

* The above is also in agreement with the literature.

From the descriptions recorded, it was often difficult to classify a case (Table 5). Most of the cases were described as having many cysts. It could well be that the presence of a few scattered small cysts is actually more frequent in autopsy material than has been reported.

Contents of cysts consisted of clear fluid in all cases. This finding is true of practically all reported cases. Occasional cysts contain blood or have suppurated, but primarily they contain clear serous fluid.

Meyenburg's complexes were present in 29 cases, absent in 41 cases. Their presence was determined by examining the microscopic sections in the files. In most cases a single representative section of the liver was found. Had multiple blocks of liver been examined, it is possible that these complexes would have been found more frequently. This may also be true for solitary cysts of the liver (nonparasitic), which most authors believe is a minimal phase of polycystic liver.

Bile ducts, gall bladder, blood vessels, and liver parenchyma were recorded as normal in all cases. These findings are in agreement with practically all reports of cases.

There was no evidence of liver dysfunction in any case, also in agreement with the literature.

A clinical diagnosis was not made in 69 cases and was made in 1 case, by peritoneoscopy. Except for rare cases encountered in the course of celiotomy, practically all the cases of polycystic liver reported in the literature were encountered as incidental findings at autopsy. Occasional authors suggest that in patients with polycystic kidneys an enlarged nodular liver should be suspected as being polycystic.

There was no specific treatment for polycystic liver in any of the above cases. Occasional reports have appeared of the use of sclerosing solutions to obliterate liver cysts. Suppurating cysts have been opened and drained. Cysts obstructing extrahepatic bile have been drained, with resulting relief of obstructive jaundice.

Aside from uremia due to polycystic kidneys, the diseases affecting these patients were of the same types as would be expected among older persons (Table 8).

The association with polycystic kidneys, and to a less extent with multiple cysts in the pancreas, lung, and other organs, is one of the impressive features of this disease (Table 9). Norris and Tyson [6] draw analogies between the noninvolution of intrahepatic bile ducts in polycystic liver and the noninvolution of embryonal nephron seg-

TABLE 8.—*Major Causes of Death*

	Cases, No.
Uremia due to polycystic kidneys	24
Myocardial infarction	14
Complications of malignant tumors	13
Cerebral hemorrhage	9
Pulmonary tuberculosis	7
Lobar pneumonia	5
Prostatic hypertrophy and uremia	5
Congestive heart failure	5
Renal tuberculosis	3
Mesenteric thrombosis	2
Ruptured aortic aneurysm	2
Appendicitis with perforation	2
Meningitis, lung abscess, malignant endocarditis, diabetic acidosis	1 each

TABLE 9.—*Incidence of Other Cysts*

	Cases, No.
Polycystic kidneys	35
Polycystic pancreas	1
Cyst of parathyroid gland	1
Cyst of pineal body	1
Cyst of peritoneum	1
Cyst of renal pelvis	1

TABLE 10.—*Incidence of Tumors*

Type	Cases, No.
Benign *	8
Malignant †	17

* Polyp of stomach, colon; fibroma of larynx, skin; adenoma of adrenal gland, kidney; myoma of stomach.
† Carcinoma of cervix, skin, lung, pancreas, esophagus, stomach, colon; meningioma; chronic lymphatic leukemia.

TABLE 11.—*Incidence of Other Congenital Anomalies*

	Cases, No.
Diverticulum (of esophagus, jejunum, colon, urinary bladder)	10
Aneurysm of cerebral arteries	4
Double ureter	2
Horseshoe kidney	1
Interventricular septal defect	1
Truncus arteriosus	1
Aneurysm of coronary artery	1
Bicuspid aortic valve	1
Separation of common bile duct and pancreatic duct	1

ments in polycystic kidney. It is unknown whether factors such as enzyme patterns or chemical "organizers" are present in these tissues which fix the embryonal structures and prevent their involution.

The incidence of congenital anomalies and of tumors in these cases (Table 10) is higher than in the general population, in comparable age groups.

Of special interest is the finding of aneurysms of the cerebral arteries in four cases (Table 11). Polycystic kidneys were also present in all four cases. A total of 47 cases of polycystic kidneys and associated intracranial aneurysms have been recorded in the literature (Poutasse and associates [18]). The 4 cases reported here make a total of 51 cases, and they are associated, in addition, with polycystic liver.

REPORT OF CASES

CASE 1.—E. D., a one-month-old Negro boy, was brought into the Los Angeles County General Hospital suffering from a severe upper respiratory infection of two days' duration. On the day of entry he suddenly became severely dyspneic and cyanotic and was rushed to the hospital. He was semicomatous on entry; the temperature was 98.6 F., pulse rate 150, and respiratory rate 72 per minute. He appeared well developed and well nourished but dyspneic and cyanotic. Numerous rales were heard in the lung fields. The heart was enlarged to percussion, and there was a loud systolic murmur over the entire precordium. The liver was palpable 2 fingerbreadths below the costal margin. The urine contained albumin. The hemoglobin was 15.5 gm. per 100 ml. of blood; the white cell count was 3,600, with 38% polymorphonuclear leucocytes, 56% lymphocytes, and 6% monocytes. X-ray examination of the chest disclosed an enlarged cardiac shadow compatible with congenital heart disease. The infant was temporarily benefited by oxygen and antibiotic therapy but died three days later.

Autopsy (#48715).—Postmortem examination disclosed a number of congenital malformations. The heart was the site of a large interventricular septal defect. Overriding this defect was a truncus arteriosus originating above fused aortic and pulmonic valves; 1 cm. distal the truncus communis separated into aorta and pulmonary trunk. The kidneys were polycystic and were fused at the lower poles, forming a very rare type of polycystic horseshoe kidney. The immediate cause of death was extensive confluent bronchopneumonia.

Examination of the Liver.—The liver weighed 70 gm. and appeared normal in shape. The surface was smooth and red-brown in color. The cut surfaces were similar in color. No cysts were visible on gross examination, but the lobular markings on the cut surfaces appeared to be much accentuated. The gall bladder, extrahepatic biliary system, and blood vessels were all normal.

Microscopic examination of the liver disclosed that the accentuation of the lobular markings resulted from the presence of numerous small intrahepatic bile ducts, some of which are dilated and cystic (Figs. 3 and 4). It is not uncommon in routine autopsy material from premature and even full-term newborn infants to find a few persisting intrahepatic bile ducts, but at the age of one month they are rarely found. The first impression obtained from the liver of this infant is that one is viewing sections of embryonic liver; yet at no stage in the embryo can this degree of intrahepatic bile duct formation be found.

Everywhere in this liver are seen various stages of formation of small intrahepatic bile ducts from liver cell cords (Fig. 5). The newly formed bile ducts have frequently grown toward the portal septa and have become separated from the liver cell cords of origin. The bile capillary lumina therefore no longer remain in continuity with these bile duct lumina, and the latter do not contain bile. In many areas the newly formed bile ducts reach to the portal septa and fuse with the interlobular bile ducts in that location (Fig. 6). In general, the newly formed bile ducts are at first tubular and later oval to round, but frequently their outlines are irregular and in the larger portal septa are occasionally bizarre. They are lined by cuboidal epithelium, the nuclei of which are uniform. Only rare normal-appearing mitoses are encountered. The nuclei are pale-staining, vesicular, and generally without nucleoli, in contrast to the nuclei of the liver cells, which are larger and more deeply staining and have prominent nucleoli.

Mallory's and Bensley's stains for mitochondria reveal none in the intrahepatic bile duct epithelium, but they are present in

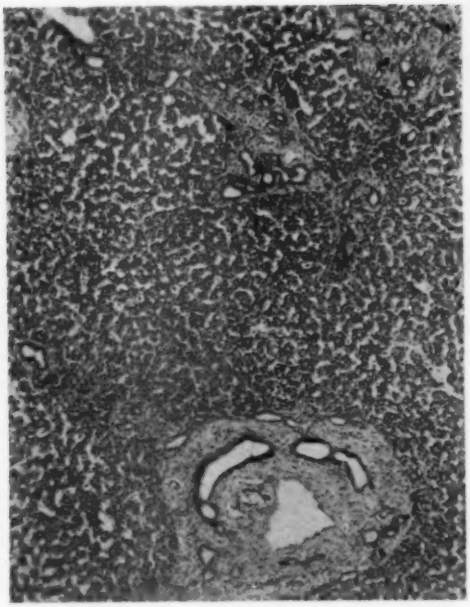
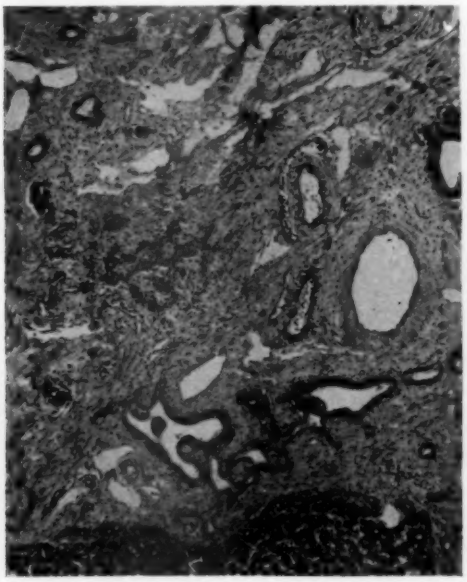


Fig. 3 (Case 1).—Liver. Numerous intrahepatic bile ducts are seen in the liver lobules, growing toward and into the portal septa. Periodic acid-Schiff stain; reduced ¼ from mag. × 50.

Fig. 4 (Case 1).—Liver. A large portal septum containing irregular groups of proliferating intrahepatic bile ducts, growing out from liver lobules and fusing with interlobular bile ducts in portal septa. Mallory's mitochondria stain; reduced ¼ from mag. × 50.



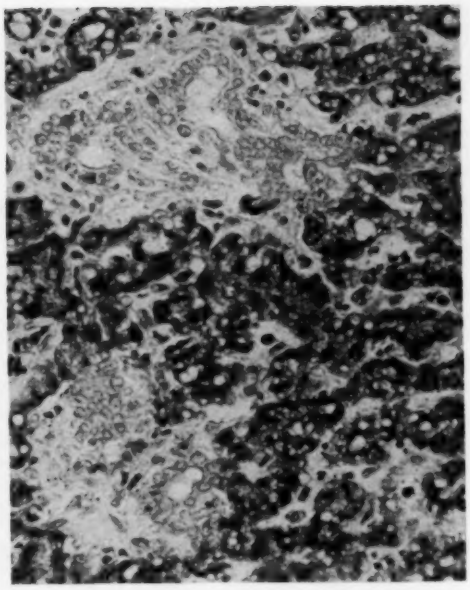


Fig. 5 (Case 1).—Groups of intralobular bile ducts are seen still attached to liver cell cords; the cytoplasm of the latter contain abundant mitochondria, which differentiates them clearly from the bile ducts. Mallory's mitochondria stain; reduced ¼ from mag. × 300.

Fig. 6 (Case 1).—Intrahepatic bile ducts are seen growing toward, and about to fuse with, branches of the interlobular bile ducts in the portal septa; the latter are differentiated by Schiff-positive material in their epithelial lining. Periodic acid-Schiff stain; reduced ¼ from mag. × 300.

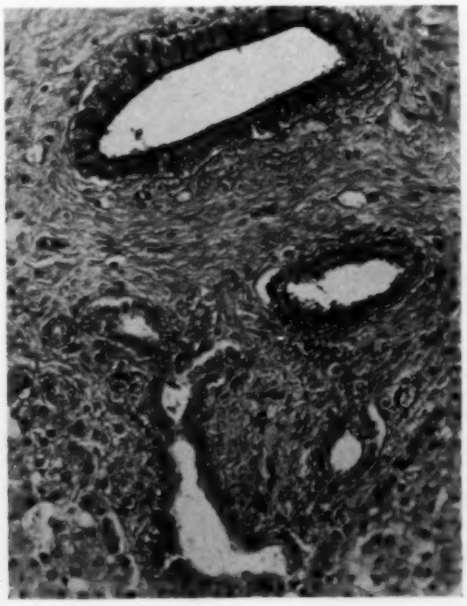


Fig. 7 (Case 2).—Sectioned surface of the liver disclosing uniform mottling by narrow light gray branched and stellate strands. Occasional small cysts up to 5 mm. in diameter are seen.

Fig. 8 (Case 1).—Liver. General view showing cysts and revealing the strands that were seen on gross examination to be composed of extremely large Meyenburg complexes, consisting of large groups of proliferating intrahepatic bile ducts. Hematoxylin and eosin; reduced ¼ from mag. × 20.

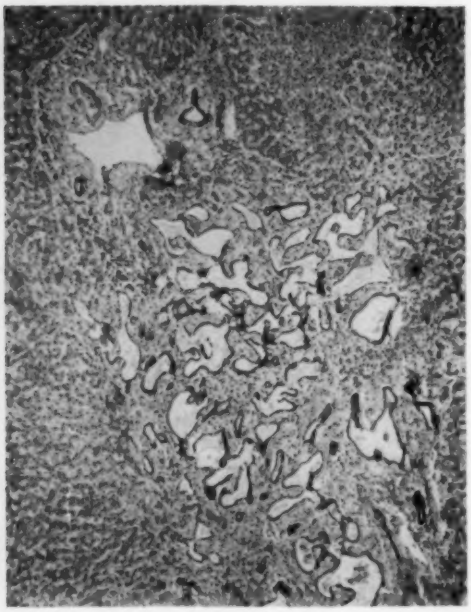
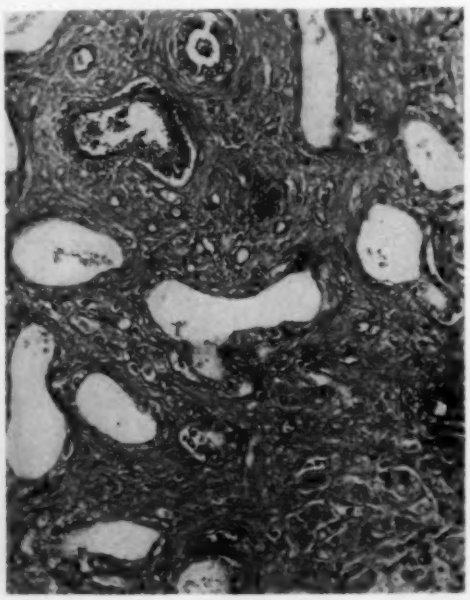


Fig. 9 (Case 2).—Low-power view revealing an extremely large Meyenburg complex, adjacent to a portal septum and composed of intrahepatic bile duct elements being formed from liver cell cords. The liver in general is the site of passive congestion. Hematoxylin and eosin; reduced ¼ from mag. × 50.

Fig. 10 (Case 2).—The proliferating intrahepatic bile ducts are grouped in relation to interlobular bile duct branches in the portal septa. Periodic acid-Schiff stain; reduced ¼ from mag. × 200.



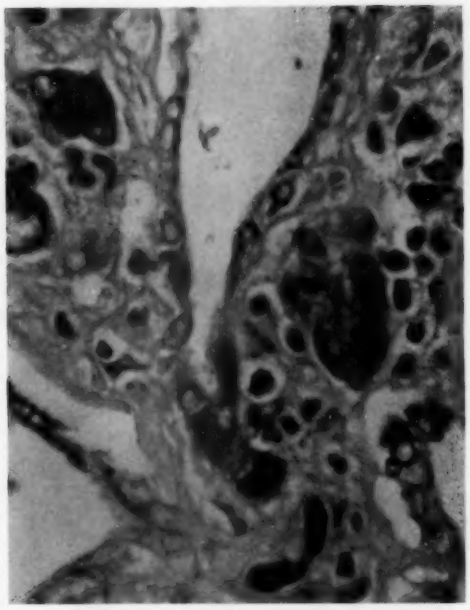


Fig. 11 (Case 2).—A newly formed intrahepatic bile duct can be seen emerging from a liver cell cord. As the bile duct epithelium differentiates from the liver cells, the mitochondria gradually disappear. Bensley's mitochondria stain; reduced ¼ from mag. × 500.

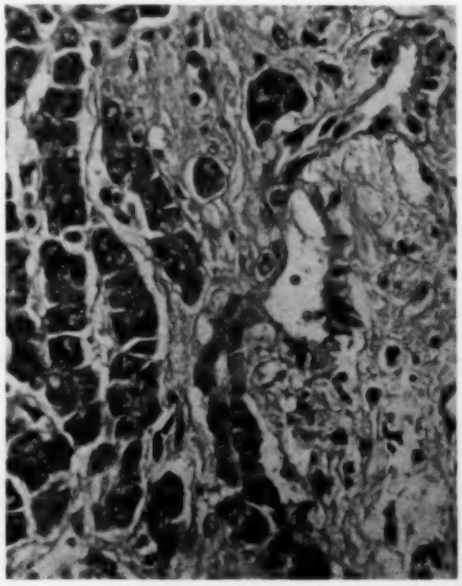
abundance in the liver cells. Periodic acid-Schiff stains reveal Schiff-positive material in the interlobular bile duct epithelium but none in the intrahepatic bile ducts. This stain sharply differentiates them even at the point of fusion. They can be further differentiated because the interlobular bile ducts are lined by more differentiated and higher cuboidal epithelium containing larger, generally oval nuclei. Altmann's stain reveals cell granules only in the interlobular bile ducts.

Case 2.—E. R., a 59-year-old white woman, entered the Los Angeles County General Hospital on Aug. 23, 1952, after two weeks of edema of the ankles and after suffering numerous severe attacks of bronchial asthma for several years. During the few weeks before entrance to the hospital she had received 0.2 mg. of digitoxin daily, as well as occasional mercurial diuretics. Physical examination disclosed a very dyspneic and cyanotic well-developed white woman with marked edema of the lower extremities; anasarca of the lower abdominal wall; an enlarged tender liver, palpable 4 finger-breadths below the costal margin, and ascites. Rhonchi and wheezing were heard throughout both lung fields; the chest was moderately barrel shaped. The heart tones were distant but regular, with no murmurs. The pulse rate was 100 per minute and regular; temperature 98 F.; respirations 24; blood pressure 110/60. The urine had a specific gravity of 1.030 and contained albumin but no casts. The peripheral blood was essentially normal. In spite of digitalization and supportive measures, she died within a short time. The clinical diagnosis was congestive heart failure due to bronchial asthma.

Autopsy (#47464).—The essential findings were markedly emphysematous lungs, which on microscopic examination presented the characteristic findings of bronchial asthma; marked hypertrophy and dilatation of the right ventricle of the heart, and severe chronic passive congestion of the liver, spleen, and kidneys.

Examination of the Liver.—The liver weighed 1,560 gm. and was normal in configuration. Its external surface was smooth and dark red-brown and was mottled everywhere with narrow light gray to yellow-gray firm strands from 1 to 3 mm. in width. On cutting, these strands were found scattered diffusely throughout the liver parenchyma

Fig. 12 (Case 2).—An elongated intrahepatic bile duct can be seen to have been formed from a liver cell cord. Its more distal portion is about to segment and to become separated. Its proximal portion is still attached to the liver cell cord. The end of the cord of liver cells has a widened bile capillary lumen, but the lumen does not remain in continuity with the bile duct. The mitrochondria of the liver cell cords are gradually reduced in number as the bile duct epithelium is differentiated from them. Mallory's mitochondria stain; reduced ¼ from mag. × 400.



(Fig. 7); they were most abundant in the major portion of the right lobe. They often were branched or stellate. Scattered among these structures were occasional thin-walled cysts, measuring up to 5 or 6 mm. in diameter, with smooth inner linings; the cysts contained clear fluid. The liver parenchyma between the gray strands was essentially normal, with distinct lobulation owing to severe chronic passive congestion. The gall bladder, bile ducts, and blood vessels were not remarkable.

Microscopic examination of the liver revealed the gray strands described above to be enormous groups of small bile ducts embedded in fibrous tissue (Fig. 8) which represent Meyenburg's complexes of great size

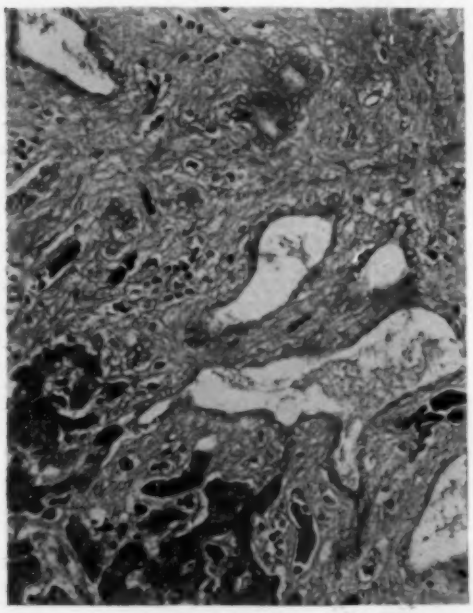


Fig. 13 (Case 2).—Liver. Intrahepatic bile ducts newly formed from liver cell cords, fusing together and growing toward portal septa. Mallory's mitochondria stain; reduced ¼ from mag. × 300.

(Fig. 9). These bile ducts resemble the intrahepatic bile ducts seen in the liver of Case 1. They are lined by low-cuboidal epithelium, the nuclei of which are pale-staining, generally without nucleoli, vesicular, and even; there is no pleomorphism, and only occasional normal-appearing mitoses are encountered. The nuclei of the liver cells are larger are more deeply staining, and have prominent nucleoli. The interlobular bile

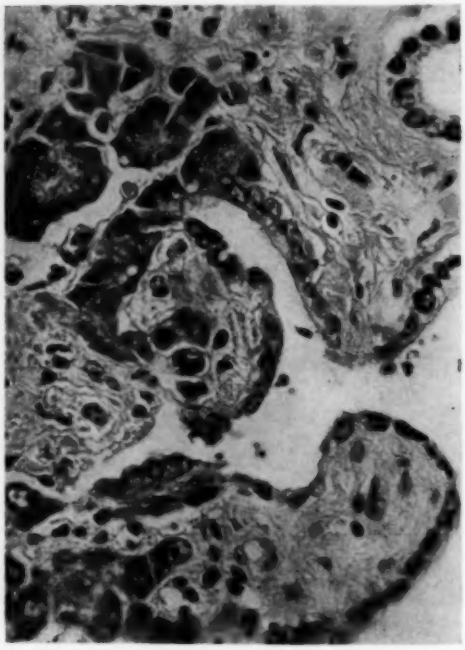
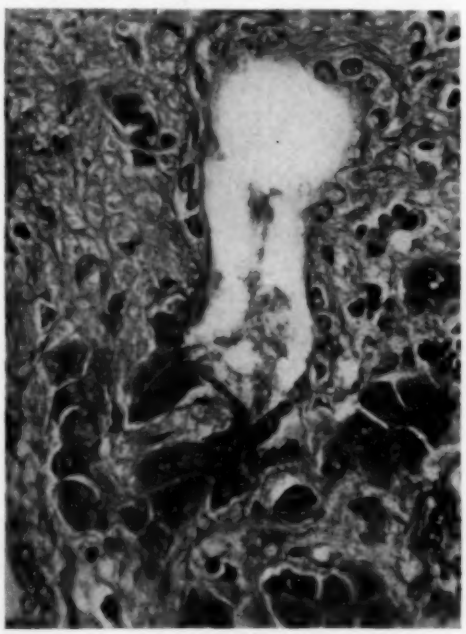


Fig. 14 (Case 2).—Liver. Fusion of several newly formed intrahepatic bile ducts. Mallory's mitochondria stain; reduced ¼ from mag. × 400.

Fig. 15 (Case 2).—Liver. Formation of intrahepatic bile ducts derived from liver cell cords. Altmann's stain; reduced ¼ from mag. × 400.



ducts in the portal septa can also be easily differentiated by their larger, often oval, more deeply staining nuclei. These large collections of intrahepatic bile ducts are always oriented toward, or joined to, the portal septa, and they cluster around the interlobular bile ducts and their branches (Fig. 10).

At the peripheral borders of these collections of bile ducts, adjacent to the liver parenchyma, numbers of these ducts can be seen being formed from the ends of the liver cell cords. The patterns that they assume in this location consist of outgrowths of elongated, rather narrow bile duct channels (Fig. 11). After separation from the liver cell cords they may segment (Fig. 12). Occasionally two or three adjacent ones appear to have fused together (Figs. 13 to 15). They then become incorporated into the large Meyenburg complexes, where they tend to widen and to become oval or round, although generally somewhat irregular; in this location they appear to continue to proliferate.

Special stains aided in identifying the various stages in the process. Mallory's as well as Bensley's stains for mitochondria revealed an abundance of mitochondria in the liver cells but none in the bile duct epithelium. At their junctions there was often a gradual diminution in mitochondria in the liver cells over a length of about five or six cells in the liver cell cord, but in other areas there was often an abrupt transition. The latter appeared to be a second stage, because in the areas of abrupt transition there was no communication between the bile duct lumen and the bile capillary of the adjacent liver cell cord; the latter appeared to be pinched off. This explains the absence of bile in these ducts and in the cysts formed from them. The periodic acid-Schiff method reveals Schiff-positive material in the interlobular bile ducts but none in the newly proliferated ducts. The latter stain with Eppinger's bile capillary method, but the interlobular bile ducts do not take this stain. Altmann's stain revealed cell granules only in the interlobular bile ducts.

SUMMARY

In the autopsy records of the Los Angeles County General Hospital over a 36-year period there were found 70 cases of polycystic liver, an incidence of 1 per 687 autopsies. The condition is increasingly frequent with advancing age; it results from the gradual cystic dilatation of groups of persisting intralobular bile ducts (Meyenburg's complexes) which fail to involute in the later embryologic development of the liver. There is a high incidence of association with bilateral congenital polycystic kidneys and occasionally with cysts of the pancreas, lungs, and other organs. There is also a high incidence of association with other congenital anomalies and with benign and malignant tumors. In four cases polycystic kidneys were associated with intracranial aneurysm. In no case was there distinct clinical or laboratory evidence of liver dysfunction; the clinical diagnosis was made in one case, by peritoneoscopy. The liver parenchyma in general, as well as the gall bladder and the extrahepatic bile ducts, are normal.

Two cases in this series are of special interest because there is seen new-formation of intrahepatic bile ducts from liver cell cords, as occurs in embryonic livers; the newly formed bile ducts are seen in various stages of cystic dilatation. The process appears to be on the border line of neoplasia, but it retains an organoid pattern and a regular arrangement. This rare finding has only once been previously reported in the literature. A review of the literature of polycystic liver is presented.

REFERENCES

1. Moschcowitz, E.: Non-Parasitic Cysts (Congenital) of the Liver, with a Study of Aberrant Bile Ducts, Am. J. M. Sc. **131**:674, 1906.

2. Lewis, F. T.: The Development of the Liver, in Keibel, F., and Mall, F. P., Editors: Manual of Human Embryology, Philadelphia, J. B. Lippincott Company, 1912, Vol. 2, pp. 403-428.

3. von Meyenburg, H.: Über die Cystenleber, Beitr. path. Anat. **64**:477, 1918.

4. Kaufmann, E.: Pathology for Students and Practitioners, translated by S. P. Reiman, New York, Blakiston Company, 1929, Vol. 2, pp. 955-957.

5. Hanser, R.: Missbildungen der Leber, in Handbuch der speziellen pathologischen Anatomie und Histologie, edited by F. Henke and O. Lubarsch, Berlin, Springer-Verlag, 1930, Vol. 5, Pt. 1, pp. 1-70.

6. Norris, R. F., and Tyson, R. M.: Pathogenesis of Polycystic Livers: Reconstructions of Cystic Elements in 2 Cases, Am. J. Path. **23**:201, 1947.

7. Bloom, W.: Embryogenesis of Human Bile Capillaries and Ducts, Am. J. Anat. **36**:451, 1926.

8. Streeter, G. L.: Developmental Horizons in Human Embryos, Publication 575, Carnegie Institution of Washington, Washington, D. C., Contrib. Embryol. **32**:162-163 and 177-181, 1948.

9. Hammar, J. A.: Über die erste Entstehung der nicht kapillaren intrahepatischen Gallengange beim Menschen, Ztschr. Mikr. anat. Forsch. **5**:59, 1926.

10. Wackerle, L.: Zur Frage der Cystenleber, Arch. path. Anat. **262**:508, 1926.

11. Herxheimer, G., and Thölldte, M.: Regeneration und Hypertrophie (Hyperplasie) der Leber, in Handbuch der speziellen pathologischen Anatomie und Histologie, edited by F. Henke, and O. Lubarsch, Berlin, Springer-Verlag, 1930, Vol. 5, Pt. 1, pp. 988-1037.

12. MacMahon, H. E., and Thannhauser, S. J.: Congenital Dysplasia of the Interlobular Bile Ducts with Extensive Skin Xanthomata, Gastroenterology **21**:488, 1952.

13. Edmondson, H. A., and Steiner, P. E.: Primary Carcinoma of the Liver: Study of 100 Cases Among 48,900 Necropsies, Cancer **7**:462, 1954.

14. Berman, C.: Primary Carcinoma of the Liver, London, H. K. Lewis & Co., Ltd., 1951, p. 68.

15. Blum, M., and Müller, W.: Ein Beitrag zur Klinik und pathologischen Anatomie der Cystenleber, Deutsches Arch. klin. Med. **173**:206, 1932.

16. Davis, C. R.: Non-Parasitic Cysts of Liver, Am. J. Surg. **35**:590, 1937.

17. Comfort, M. W.; Gray, H. K.; Dahlin, D. C., and Whitesell, F. B., Jr.: Polycystic Disease of the Liver: Study of 24 Cases, Gastroenterology **20**:60, 1952.

18. Poutasse, E. F.; Gardner, W. J., and McCormack, L. J.: Polycystic Kidney Disease and Intracranial Aneurysm, J. A. M. A. **154**:741, 1954.

News and Comment

ANNOUNCEMENT

Cancer Research Symposium.—The Ninth Annual M. D. Anderson Symposium on Fundamental Cancer Research will be held on March 10-12, 1955, at the University of Texas M. D. Anderson Hospital and Tumor Institute in the Texas Medical Center, Houston. Dr. George Gomori, of the University of Chicago, will be chairman of a program devoted to histochemistry. On the evening of March 11, the Bertner Award for outstanding contributions in the field of cancer research will be made.

Conference on Protein Metabolism.—The 11th Annual Conference on Protein Metabolism, "Some Physiological Aspects and Consequences of Parasitism," sponsored by the Bureau of Biological Research of Rutgers University, State University of New Jersey, will be held in New Brunswick, N. J., Jan. 28 and 29, 1955. Reports will be given on cultivation of intracellular parasites, intestinal physiology and the host-parasite relationship, protein metabolism of intracellular parasites, glycolytic enzymes of schistosomes, antibody formation and leishmaniasis, and metabolism of the host. The conference is open to all registrants. Reservation blanks may be secured from William H. Cole, Ph.D., Rutgers University.

AORTIC LESIONS INDUCED IN THE BIRD BY DIETHYLSTILBESTROL INJECTIONS AND CHOLESTEROL FEEDING

A Study of Their Development and Regression

STUART LINDSAY, M.D., San Francisco
C. W. NICHOLS Jr., M.A.
and
I. L. CHAIKOFF, M.D., Berkeley, Calif.

PREVIOUS studies have shown that arteriosclerosis occurs naturally in the bird. The character of the aortic lesion varies with the portion of the vessel involved and with the sex of the animal. The lesion in the elastic thoracic aorta is more prominent in the female bird than in the male and consists (in the earliest stages) of an accumulation of lipid in the intima and media, followed by intimal fibrous thickening. The naturally occurring lesion in the muscular abdominal aorta is primarily fibrotic in nature and is seen most frequently in male birds.[1]

Lipemia of either exogenous (cholesterol feeding) or endogenous (diethylstilbestrol injection) origin causes lipid infiltration of the vascular wall. This infiltration may produce a new atheromatous lesion or may modify an existing spontaneous lesion. In the abdominal aorta lipid deposition becomes pronounced in the preexisting intimal plaques. Experimentally induced aortic disease and that occurring naturally differ only in the degree of intimal thickening and in the amounts and proportions of the various lipids deposited. The lesions induced by diethylstilbestrol injections more closely resemble the spontaneous lesion than do those resulting from cholesterol feeding. It has been pointed out that in the cholesterol-fed bird the deposition of lipid in the arterial wall is a single manifestation of a generalized cholesterol deposition.[1]

Aided by grants from the U. S. Public Health Service and the Life Insurance Medical Research Fund.

From the Departments of Pathology (San Francisco) and Physiology (Berkeley) of the University of California School of Medicine.

EXPERIMENTAL

Single-comb, white Leghorn cockerels, 3 to 4 months old, obtained from the Poultry Division of this university, were divided into the following three groups: I, 35 controls; II, 49 diethylstilbestrol-injected birds, and III, 15 cholesterol-fed birds.

Throughout the entire experimental period the birds of Groups I and II were fed Purina broiler chow to which had been added 5% cottonseed (Wesson) oil.

Two pellets, each containing 12 mg. of diethylstilbestrol, were implanted subcutaneously into each bird of Group II at monthly intervals for the periods shown in Table 4.

The birds of Group III were fed Purina broiler chow to which had been added 2% cholesterol and 5% cottonseed oil, for periods recorded in Table 5.

A number of birds of Group II were killed while still under the influence of diethylstilbestrol treatments. Other birds of this group, which had been under diethylstilbestrol treatments for 10 months, were killed at bimonthly intervals up to 12 months after cessation of diethylstilbestrol injections. However, pellet residues were removed from these birds at the end of the 10-month period.

A number of birds of Group III were killed while still being fed the cholesterol-containing diet. Other birds of this group were killed at bimonthly intervals up to eight months after cholesterol feeding had been discontinued. During this latter eight-month period the birds were fed the Purina broiler chow to which had been added 5% cottonseed oil.

At monthly intervals the birds were weighed and blood samples were taken from an alar vein. Plasma was analyzed for total cholesterol and fatty acids by procedures described elsewhere.*

RESULTS

The results are recorded in Tables 1 and 2. The high lipid values observed in birds fed cholesterol and implanted with diethylstil-

* References 2 and 3.

bestrol are in keeping with those noted in an earlier report.[1] Upon removal of the diethylstilbestrol implants or cessation of cholesterol feeding, the concentrations of lipids in plasma returned to normal.

The birds were killed by exsanguination, and the hearts and aortas were removed. The aortas were opened longitudinally, and the gross appearance of the intimas was noted. The tissues were fixed in 10% formaldehyde U. S. P. Sectioning of the tissues and microscopic grading were done in the same manner as described previously.[1] A number of the birds died spontaneously during the course of the experiment, and their tissues were also treated as described above.

Although designed primarily to study the the question of regression of arteriosclerosis

TABLE 1.—*Total Cholesterol and Total Fatty Acids in Plasma of Cholesterol-Fed and Diethylstilbestrol-Implanted Birds*

Interval, Mo.	Total Cholesterol, Mg. %				Total Fatty Acids, Mg. %			
	Ave.	Range	SE$\bar{x}$*	N	Ave.	Range	SE$\bar{x}$	N
				Diethylstilbestrol-Implanted Birds				
1	140	62-415	16	26	941	194-6,850	269	26
2	606	71-1,270	56	33	1,900	286-15,800	804	33
3	1,006	546-1,400	45	27	13,494	7,410-18,600	666	27
4	1,106	475-1,570	148	7	10,967	770-17,800	222	7
5	1,025	173-1,400	58	28	12,700	194-18,900	912	28
6	873	175-1,300	145	8	10,874	1,520-16,900	236	7
7	902	96-1,590	228	8	9,530	382-15,100	242	7
8	915	102-1,510	144	13	12,196	239-17,900	180	13
9	904	120-1,560	81	24	9,128	596-20,100	109	24
10	1,267	845-1,700	71	12	14,387	4,140-20,600	123	12
	Diethylstilbestrol implants stopped							
11	.....	.....	...	..	.....	.....	...	..
0.5	118	69-204	13	11	323	172-814	60	10
1	100	64-159	9	12	260	166-415	78	13
2	86	73-118	32	7	225	169-354	85	7
8	90	82-98	5	3	...	.....	...	..
10	54	47-56	3	3	253	195-292	30	3
				Cholesterol-Fed Birds				
1	547	247-1,200	153	7	1,106	520-1,600	130	7
2	579	206-1,730	116	14	850	385-2,100	126	14
3	564	175-1,630	147	10	562	225-1,360	114	10
4	664	256-978	161	4	703	390-978	143	4
5	286	167-454	86	3	285	239-354	118	3
6	439	189-750	72	7	454	277-743	60	7
7	416	125-892	175	4	349	195-569	91	4
8	236	111-360	27	10	304	191-439	29	9
9	281	119-534	128	3	360	198-575	112	3
10	340	158-632	49	10	386	242-663	45	10
11	365	117-906	89	10	375	196-828	74	10
	Cholesterol feeding stopped							
0.5	136	72-185	15	7	235	189-332	20	7
1	97	82-111	8	3	314	240-389	74	2
2	84	79-90	6	2	230	199-262	32	2
8	...	.....	...	..	...	.....	...	..
10	...	.....	...	..	...	.....	...	..

* Standard error of the mean derived from the formula $SE\bar{x} = \frac{S}{\sqrt{N}}$.

TABLE 2.—*Total Cholesterol and Total Fatty Acids in Plasma of Control Birds Determined at Infrequent Intervals During the Experiment*

Total Cholesterol, Mg. %				Total Fatty Acids, Mg. %			
Ave.	Range	SE$\bar{x}$	N	Ave.	Range	SE$\bar{x}$	N
100	60-173	4	35	227	121-454	12	34

in the bird, the present work has also served to clarify the developmental aspects of the disease. The latter will be dealt with first.

1. *Development.* — Naturally Occurring Arteriosclerosis (Table 3): In the control birds, two types of arteriosclerosis were found. One consisted primarily of lipid infiltration, first of the media and later of the intima, of the thoracic aorta (Fig. 1). In only one of 35 birds was a grossly visible intimal lesion found. Only two of the birds displayed thickening of the intima, visible microscopically, as a result of lipid infiltration of the vascular wall (Fig. 2). While approximately half of the birds showed lipid infiltration, none demonstrated refractile substance suggestive of cholesterol.

A second type of naturally occurring arteriosclerosis in the bird was observed in the abdominal aorta. This lesion was primarily fibrous in nature, although lipid substances appeared later as the lesion progressed. The present study shows that the initial alteration in the abdominal vessel consisted of degeneration and fragmentation of the internal elastic membrane. This injury was followed by deposition of acid mucopolysaccharide and proliferation of fibroblasts in the intima. Partial replacement of the mucoid ground substance by reticulum and collagenous fibers

TABLE 3.—*Aortic Lesions in Control Birds (Group 1)*

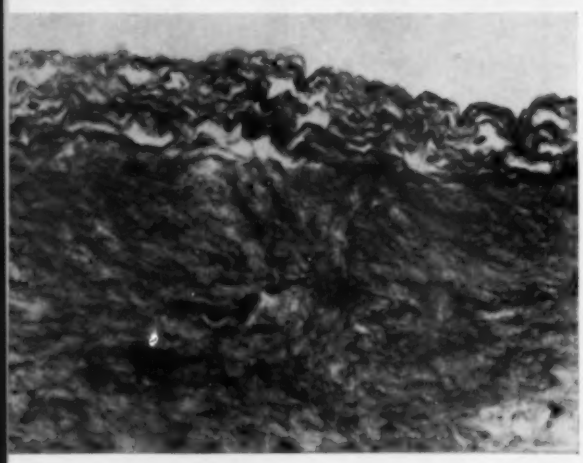
Bird*	Age, Mo.	Interval on Experiment, Mo.	Microscopic Examination of Thoracic Aorta			Microscopic Examination of Abdominal Aorta		
			Intimal Thickening†	Lipids‡	Cholesterol‡§	Intimal Thickening†	Lipids‡	Cholesterol‡§
1832 D	5	1	0	0	0	1+	1+	0
4702 K	6	3	0	0	0	1+	0	0
1161 D	8	5	0	0	0	2+	0	0
4969 D	8	5	0	0	0	2+	1+	0
1248 K	9	6	0	0	0	2+	0	0
4974 D	10	7	0	0	0	1+	0	0
4970 K	10	7	0	0	0	0	0	0
4980 D	12	9	0	0	0	2+	2+	0
1081 D	14	11	0	2+	0	1+	2+	0
4684 K	16	13	0	0	0	1+	1+	0
4975 D	16	13	0	1+	0	2+	3+	0
2188 D	17	14	0	1+	0	3+	3+	0
1102 K	17	14	0	0	0	4+	2+	?
1131 K	17	14	0	1+	0	4+	2+	1+
1250 K	17	14	0	0	0	4+	2+	0
3688 K	17	14	0	0	0	4+	2+	0
3725 K	18	15	0	0	0	3+	2+	1+
4971 K	19	16	0	2+	0	2+	3+	0
1148 K	19	16	0	1+	0	3+	3+	0
2147 D	20	16	0	1+	0	1+	0	0
1093 K	21	18	0	1+	0	2+	3+	0
5413 K	21	18	1+	2+	0	4+	2+	1+
3640 K	21	18	0	0	0	1+	0	0
1205 K	21	18	0	0	0	2+	1+	0
5349 K	21	18	0	1+	0	2+	2+	0
1106 K	22	19	0	2+	0	4+	3+	0
4972 K	22	19	0	0	0	2+	1+	0
2463 K	22	18	0	1+	0	2+	1+	0
3941 K	22	19	0	1+	0	3+	3+	0
1267 K	22	19	0	0	0	1+	0	0
1151 D	22	19	0	1+	0	2+	2+	0
4973 K	23	20	0	0	0	2+	2+	0
2659 K	24	21	0	0	0	3+	2+	0
2764 K	24	21	1+	2+	0	1+	2+	0
1887 K	24	21	0	1+	0	1+	3+	0

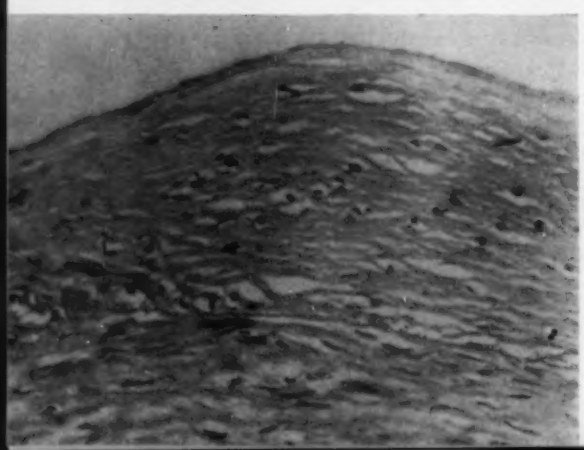
* D indicates died; K, killed.

† Microscopic grading of lesions: 1+ indicates minimal intimal thickening; 2+, mild intimal thickening; 3+, moderate intimal thickening; 4+, pronounced intimal thickening or presence of large plaques.

‡ Microscopic grading of lipids: 1+ indicates minimal amounts in intima or media; 2+, mild infiltration in intima or media; 3+, moderate infiltration mainly in the intima; 4+, large amounts mainly in the intima.

§ Estimated by polarized light.





occurred later (Figs. 3 to 5). The deposition of lipid substance, which appeared first in the deeper layers of the thickened intima, was a late phenomenon. Thus, the naturally occurring arteriosclerosis of the abdominal portion of the aorta of the bird has a pathogenesis similar to that occurring naturally in man,† dogs,‡ and cats.§ Table 3 shows that the severity of intimal thickening and the degree of lipid infiltration of the abdominal aorta tend to increase with the age of the bird. While refractile material, presumably cholesterol, may appear in the abdominal lesions, the amounts found there were small.

Diethylstilbestrol-Induced Lesions (Table 4): The thoracic lesions found in the birds injected with diethylstilbestrol closely resembled those occurring naturally, differing mainly in the amount of lipid substance deposited in the aortic wall (Figs. 6 and 7). Table 4 shows that during the 10-month period the birds were injected with diethylstilbestrol lesions of increasing severity developed in the thoracic aorta.

In the abdominal aorta of the diethylstilbestrol-injected birds, the lesions were severer than were those observed in control birds of similar ages. The earliest lesion observed in the intima of the abdominal aorta consisted of fragmentation and splitting of segments of the internal elastic membrane. These segments were surrounded by narrow masses of fibrillary acid mucopolysaccharide

† References 4 and 5.

‡ References 6 and 7.

§ Lindsay, S., and Chaikoff, I. L.: Unpublished data.

Fig. 1 (Bird 1106).—Control. Thoracic aorta showing lipid deposition in medial layer. Sudan IV-hematoxylin stain; reduced 1/9 from mag. × 160.

Fig. 2 (Bird 2764).—Control. Thoracic aorta showing thickened intima consisting of vesicular connective tissue cells. Laidlaw connective tissue stain; reduced 1/9 from mag. × 320.

Fig. 3 (Bird 2147).—Control. Abdominal aorta showing fibrous thickening of intimal layer. Note fragmentation and reduplication of internal elastic membrane. Verhoeff-Van Gieson stain; reduced 1/9 from mag. × 320.

Fig. 4 (Bird 1131).—Control. Abdominal aorta showing intimal fibrosis. Hematoxylin and eosin stain; reduced 1/9 from mag. × 320.

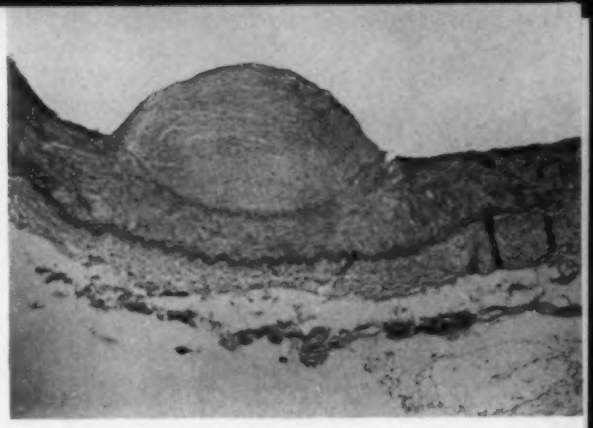
substance, which in some instances elevated the endothelium slightly (Fig. 8). No lipid material appeared in the early lesions, and there was no cellular proliferation.

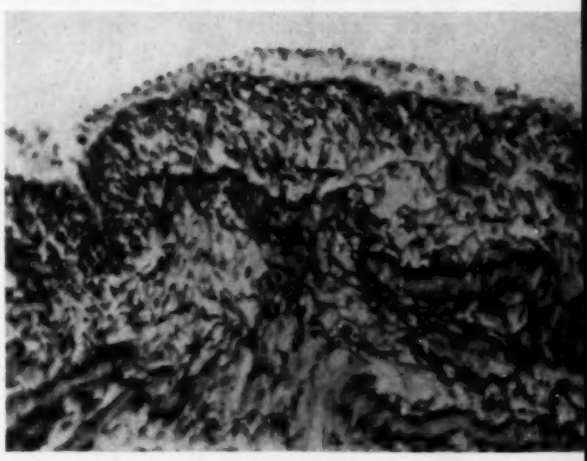
The plaques observed later in the lower portion of the abdominal aorta were ridge-like structures. In younger birds these were white, whereas in the older birds they were yellow, indicating the presence of fat.

The larger intimal plaques of the abdominal aorta consisted of fibroblasts and abundant intercellular material, both arranged parallel to the endothelial lining. The deeper portions of these plaques were composed of loosely arranged, relatively acellular, mucoid connective tissue, which was mainly mucopolysaccharide. A few undulating delicate reticulum and collagenous fibers were present. These were most numerous in the superficial and deeper portions of the plaques. Beneath these larger plaques the internal elastic membrane had partially disappeared (Fig. 9). That remaining was fragmented and reduplicated.

As compared with the control group, greater degrees of intimal thickening and lipid infiltration were observed microscopically in the abdominal aorta. Refractile material was present in the more advanced abdominal lesions of the diethylstilbestrol-injected birds but was absent in the control birds.

Cholesterol-Induced Lesions (Table 5): The thoracic lesions in the cholesterol-fed birds were fundamentally similar to those





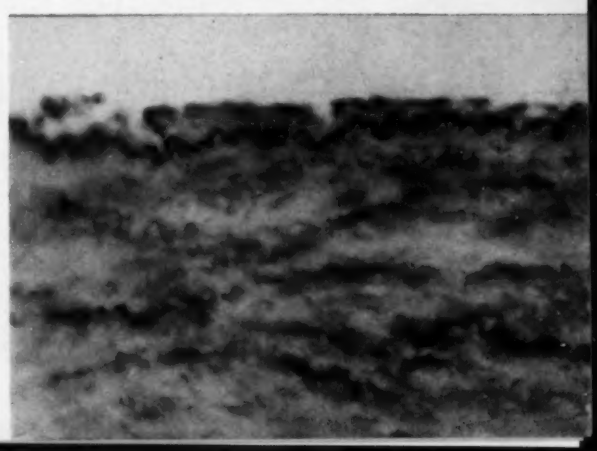


Fig. 5 (Bird 1102).—Control. Abdominal aorta with large, fibrous intimal plaque. Hematoxylin and eosin stain; reduced 1/9 from mag. × 45.

Fig. 6 (Bird 1938).—Diethylstilbestrol-injected. Thoracic aorta showing deposition of abundant lipid in inner medial layer. Nile blue stain; reduced 1/9 from mag. × 80.

Fig. 7 (Bird 1222).—Diethylstilbestrol-injected. Thoracic aorta showing early intimal thickening. The intimal cells appear to arise from endothelium. Verhoeff-Van Gieson stain; reduced 1/9 from mag. × 320.

Fig. 8 (Bird 1703).—Diethylstilbestrol-injected. Abdominal aorta showing early intimal lesion. Fragmentation of the internal elastic membrane is associated with deposits of mucopolysaccharide, shown here beneath the endothelium. Verhoeff-Van Gieson stain; reduced 1/9 from mag. × 960.

TABLE 4.—*Aortic Lesions in Diethylstilbestrol-Implanted Birds (Group II)**

Bird	Age, Mo.	Period: On Diethylstilbestrol,† Mo.	Period: Off Diethylstilbestrol, Mo.	Microscopic Examination of Thoracic Aorta: Intimal Thickening	Thoracic: Lipids	Thoracic: Cholesterol	Microscopic Examination of Abdominal Aorta: Intimal Thickening	Abdominal: Lipids	Abdominal: Cholesterol
1703 K	4	1	..	0	0	0	1+	0	0
3948 K	5	2	..	0	2+	0	0	0	0
1847 K	6	2	..	0	1+	0	1+	1+	0
1099 K	7	4	..	0	3+	1+	2+	2+	0
2879 D	7	4	..	0	2+	0	2+	1+	0
3718 K	7	4	..	0	2+	0	2+	3+	0
1566 K	8	4	..	0	3+	0	1+	3+	0
2383 K	8	4	..	0	3+	0	3+	2+	0
2299 K	8	4	..	0	2+	0	0	1+	0
5312 D	8	6	..	0	2+	0	2+	2+	0
1165 D	8	6	..	0	3+	2+	1+	2+	0
1303 D	9	6	..	0	2+	1+	2+	3+	0
5091 D	10	7	..	1+	3+	2+	3+	4+	1+
5176 K	10	6	..	1+	3+	2+	2+	3+	1+
4968 K	10	7	..	2+	2+	3+	2+	3+	2+
5392 D	11	8	..	0	2+	0	2+	2+	0
5126 D	11	7	..	1+	3+	2+	1+	2+	0
5127 D	11	7	..	2+	3+	2+	2+	4+	2+
2285 D	11	7	..	0	2+	2+	1+	2+	0
5080 K	12	8	..	2+	2+	3+	2+	2+	0
1957 K	13	10	..	0	2+	1+	4+	4+	3+
1938 K	13	10	..	1+	2+	1+	0	3+	0
5144 K	14	10	..	0	2+	1+	3+	2+	0
5161 K	14	10	..	2+	3+	2+	4+	3+	2+
5086 K	14	10	..	2+	2+	2+	1+	2+	0
5112 K	14	10	..	0	2+	0	2+	2+	0
2876 K	15	10	2	1+	2+	0	2+	2+	0
5467 K	17	10	3	3+	2+	0	2+	2+	0
3870 K	17	10	3	2+	3+	3+	3+	2+	2+
5429 D	18	10	4	0	2+	0	2+	2+	0
2193 K	18	10	4	2+	3+	0	2+	2+	0
5391 K	19	10	5	1+	2+	2+	4+	3+	2+
1815 K	20	10	6	1+	2+	0	1+	2+	0
3679 K	20	10	6	1+	0	0	3+	3+	0
5398 K	20	10	6	0	2+	0	3+	3+	0
1295 K	21	10	8	2+	2+	0	4+	3+	2+
2073 K	21	10	8	0	1+	0	4+	3+	0
3683 K	21	10	8	0	1+	0	2+	3+	2+
3647 K	22	10	8	1+	2+	0	1+	1+	0
1777 K	23	10	10	1+	2+	0	2+	2+	0
1838 K	23	10	10	2+	2+	1+	2+	2+	1+
1319 K	23	10	10	0	2+	0	4+	3+	0
1829 K	24	10	12	..	..	0	4+	2+	0
2689 K	25	10	12	0	1+	0	1+	1+	0
1722 K	25	10	12	1+	1+	0	2+	2+	0
1848 K	25	10	12	2+	2+	0	2+	3+	1+
2223 K	25	10	10	1+	..	1+	4+	4+	2+
3181 K	25	10	12	2+	1+	0	3+	2+	1+
5030 D	24	10	8	1+	2+	1+	2+	3+	0

* For explanation of symbols, see footnoes to Table 3.
† Each bird was injected subcutaneously with two pellets, each containing 12 mg. of diethylstelbestrol, at monthly intervals for the periods shown below.

TABLE 5.—*Aortic Lesions in Cholesterol-Fed Birds* *

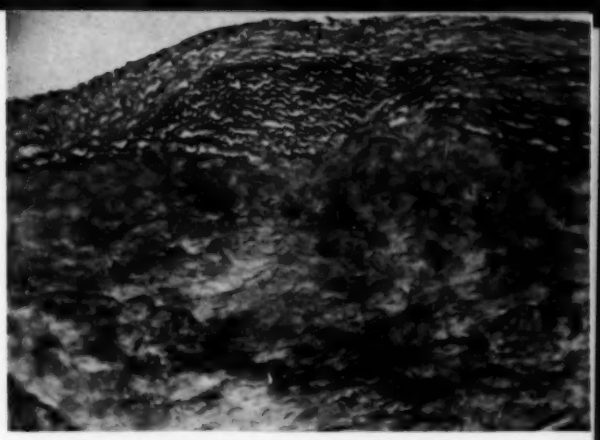
Bird	Mo. Age,	Period: On Cholesterol, Mo.	Period: Off Cholesterol, Mo.	Microscopic Examination of Thoracic Aorta: Intimal Thickening	Thoracic: Lipids	Thoracic: Cholesterol	Microscopic Examination of Abdominal Aorta: Intimal Thickening	Abdominal: Lipids	Abdominal: Cholesterol
5026 D	4	1	0	2+	2+	0	1+	0	0
1177 D	8	5	0	3+	4+	3+	3+	3+	3+
5149 K	10	7	0	2+	2+	2+	3+	3+	3+
4212 K	14	11	0	2+	2+	2+	2+	3+	2+
5135 K	14	11	0	2+	2+	0	3+	2+	0
5094 K	14	11	0	0	1+	0	3+	2+	0
5162 D	15	11	1	1+	2+	0	3+	3+	3+
4976 K	16	11	2	3+	3+	2+	4+	3+	3+
2539 D	18	10	4	2+	3+	0	3+	4+	4+
3598 K	18	10	4	1+	1+	0	3+	3+	2+
4977 K	18	11	4	1+	1+	0	3+	4+	3+
5143 D	19	11	6	2+	1+	0	3+	3+	1+
4979 K	20	11	6	0	1+	0	3+	3+	2+
5089 K	22	11	8	1+	1+	1+	2+	3+	0
5104 K	22	11	8	1+	1+	0	3+	2+	0

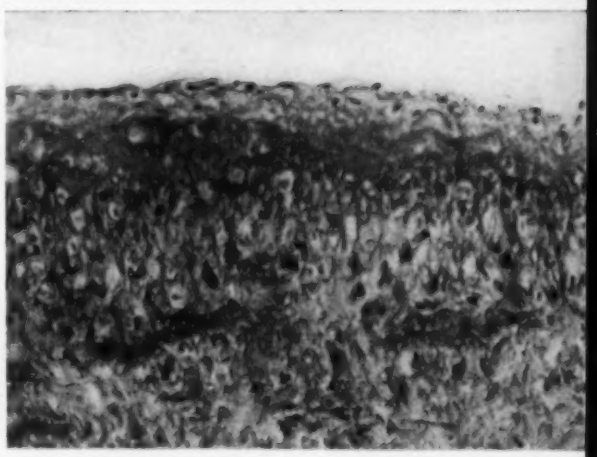
* For explanation of symbols, see footnotes to Table 3.

found naturally, as well as to those observed after injections of diethylstilbestrol. The plaques, however, were more numerous, tended to coalesce, and were tan or white rather than yellow. Table 5 shows that lipid infiltration and secondary intimal thickening were severer and appeared earlier in the cholesterol-induced lesions than in the naturally occurring lesions or in those resulting from injections of diethylstilbestrol. The amounts of refractile material (presumably cholesterol) deposited in the vascular wall of the cholesterol-fed birds were greater than those observed in the diethylstilbestrol-injected birds (Figs. 15 to 17).

The cholesterol-fed birds developed grossly visible plaques in the abdominal portions of the aorta. Under the microscope these lesions showed pronounced intimal thickening in which collections of foam cells associated with an abundance of lipid material, including refractile substance. Large cholesterol crystals and small calcific deposits were seen in the largest plaques.

2. *Regression.*—In considering the question of regression of aortic arteriosclerosis in the bird, the following two points should be noted: 1. The pathogenesis of the thoracic and abdominal lesions differs. 2. In older birds the naturally occurring lesions, particularly of the abdominal aorta, continue to progress, while the experimentally induced lesions (usually superimposed upon the





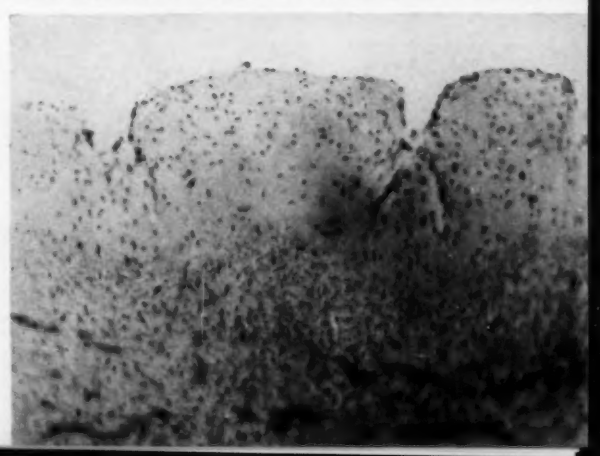
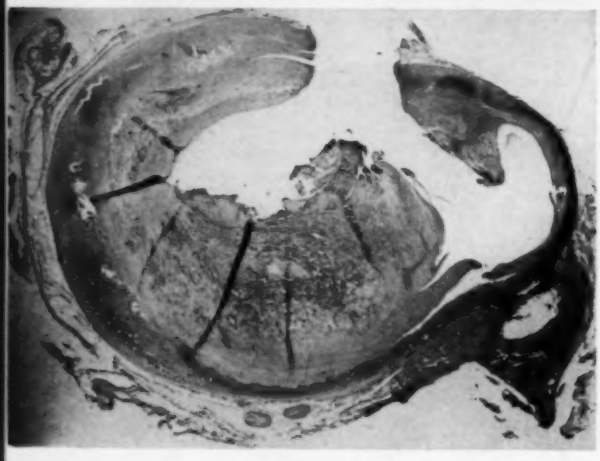


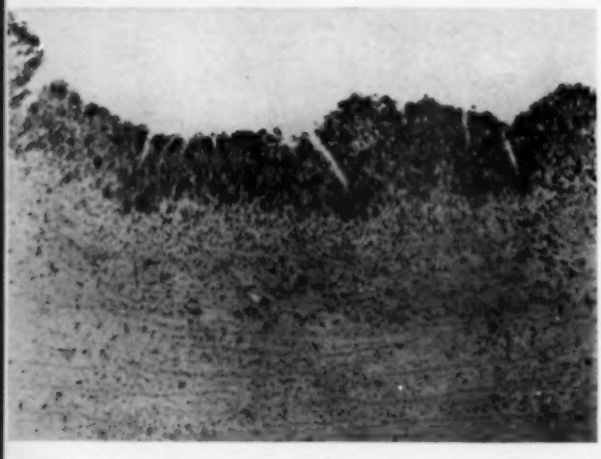
Fig. 9 (Bird 3679).—Diethylstilbestrol-injected. Abdominal aorta showing layering of thickened intima. Note loss of continuity of the internal elastic membrane. Verhoeff-Van Gieson stain; reduced 1/9 from mag. × 80.

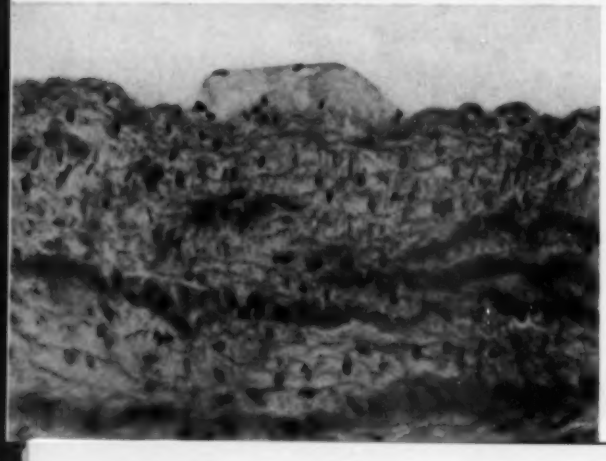
Fig. 10 (Bird 1829).—Diethylstilbestrol-injected. Thoracic aorta (postinjection period) showing thickened intima consisting mainly of acid mucopolysaccharide. Colloidal iron-Prussian blue stain; reduced 1/9 from mag. × 320.

Fig. 11 (Bird 2193).—Diethylstilbestrol-injected. Thoracic aorta (postinjection period) showing decreased cellularity of thickened intima, with condensation of intercellular fibers. Laidlaw connective tissue stain; reduced 1/9 from mag. × 320.

Fig. 12 (Bird 1829).—Diethylstilbestrol-injected. Thoracic aorta (postinjection period) showing thickened, indented intima with amorphous hyaline intercellular substance. Hematoxylin and eosin stain; reduced 1/9 from mag. × 160.







spontaneous ones) probably do not progress after the stimulus to their formation is removed.

Diethylstilbestrol-Induced Lesions (Table 4): A decrease in severity of the grossly visible thoracic aortic arteriosclerosis was observed during the 12 months after diethylstilbestrol injections were discontinued. Microscopic examination, however, revealed that the intimal thickening was as severe as before. There was a general diminution in the amounts of lipid in the thoracic aortic wall, and a few lesions still contained refractile material. The intercellular substances in the thickened intima became condensed in pericellular deposits as the lipid substance (and the cells containing it) disappeared (Figs. 10 to 12).

The lesions of the abdominal aorta displayed little or no regression after cessation of diethylstilbestrol injections. The degree of intimal thickening present at the end of the injection period persisted. In addition, the amounts of lipid material found during the 12-month period following cessation of diethylstilbestrol injections had increased. Some of the larger plaques contained large cholesterol crystals, pools of lipid-containing fluid, and calcific deposits (Fig. 13). One bird showed an extensive aneurysmal dilation of the upper abdominal aorta complicated by thrombosis (Fig. 14). None of

Fig. 13 (Bird 5391).—Diethylstilbestrol-injected. Abdominal aorta (postinjection period) showing large intimal plaque. The deeper portion is mucoid, and focal calcification is present. Hematoxylin and eosin stain; reduced 1/9 from mag. × 80.

Fig. 14 (Bird 3181).—Diethylstilbestrol-injected. Abdominal aorta (postinjection period) showing cross section of fusiform aneurysm. There is extensive destruction of the medial layer, and the lumen is partially occluded by a mural thrombus. Hematoxylin and eosin stain; reduced 1/9 from mag. × 10.

Fig. 15 (Bird 1177).—Cholesterol-fed. Thoracic aorta showing abundance of lipid in thickened intima. The denser deposits are in foam cells. Sudan IV-hematoxylin stain; reduced 1/9 from mag. × 80.

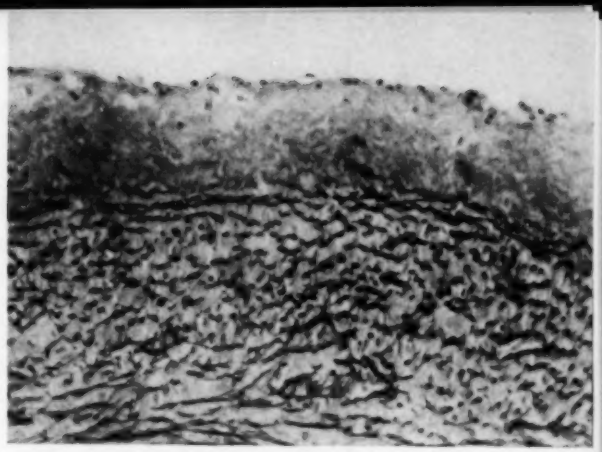
Fig. 16 (Bird 5104).—Cholesterol-fed. Thoracic aorta showing early intimal plaque composed of foam cells. These appear to have arisen from endothelium. Hematoxylin and eosin stain; reduced 1/9 from mag. × 320.

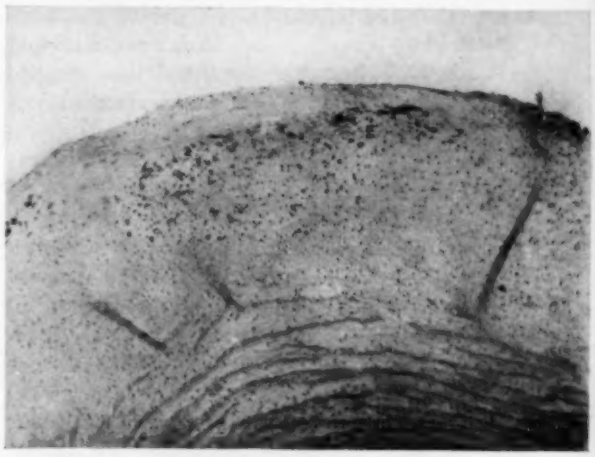
these changes was observed during the period of diethylstilbestrol injection.

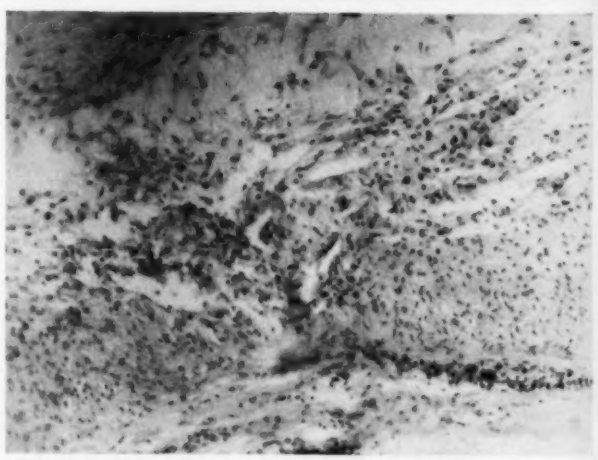
It would appear that the regressive changes in the experimentally induced arteriosclerotic lesions of the abdominal aorta are obscured by the continuing development of the naturally occurring disease in this segment of the vessel. A comparison of the lesions of the diethylstilbestrol-injected birds with those of the control birds of the same ages (Tables 3 and 4) indicates that the older control birds had lesions almost as severe as those found in the older diethylstilbestrol-injected birds during the 12-month period following cessation of diethylstilbestrol injections.

Cholesterol-Induced Lesions (Table 5): The thoracic aortic lesions, grossly visible in the cholesterol-fed birds, decreased in number and size during the eight-month period after withdrawal of cholesterol from the diet. Microscopic examination of the thoracic aortas of these birds demonstrated decreasing intimal thickening and increasing amorphous hyalinization of the intercellular stroma, which contained small, calcific deposits (Fig. 18). The degree of lipid infiltration and the amounts of refractile substances were less during the postfeeding period than during the period when the birds were ingesting cholesterol.

After the cholesterol feeding was stopped, the lesions of the abdominal aorta tended to enlarge for the next four to six months. Severe intimal disease was observed micro-







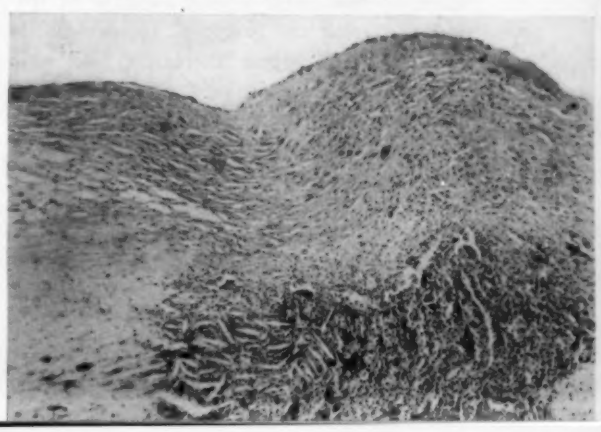


Fig. 17 (Bird 5135).—Cholesterol-fed. Thoracic aorta showing thickened, fibrous intima displaying fibrillary intercellular substance. Verhoeff-Van Gieson stain; reduced 1/9 from mag. × 160.

Fig. 18 (Bird 4976).—Cholesterol-fed. Thoracic aorta (postfeeding period) showing hyalinization of intercellular substance of thickened intima. Numerous calcific deposits are present. Hematoxylin and eosin stain; reduced 1/9 from mag. × 80.

Fig. 19 (Bird 5162).—Cholesterol-fed. Abdominal aorta (postfeeding period) showing penetration of media by foam cells originating in the intima. Hematoxylin and eosin stain; reduced 1/9 from mag. × 160.

Fig. 20 (Bird 4976).—Cholesterol-fed. Abdominal aorta (postfeeding period) showing large abdominal plaque containing deposits of cholesterol producing a foreign body reaction. Hematoxylin and eosin stain; reduced 1/9 from mag. × 80.

scopically. Groups of foam cells often extended into the medial layer (Fig. 19). The large plaques retained abundant lipid substance. Considerable amounts of refractile material remained, including large cholesterol crystals, which were more numerous at this time than during the feeding period. These crystals were often surrounded by numerous foreign-body giant cells (Fig. 20). Large and small calcific deposits were more plentiful in the intimal plaques during this post-feeding period. In two birds examined eight months after cholesterol had been omitted from the diet, no refractile material was found in the intimal plaques.

COMMENT

1. *Published Observations on Regression of Arteriosclerosis.*—The question of regression of arteriosclerosis has received some attention. The earliest studies were those of Anitschkow [8] and others,[9] who observed regression in cholesterol-induced aortic lesions in the rabbit following cessation of cholesterol feeding. It was found that the less advanced lesions regressed more completely. Lipids gradually diminished in quantity but anistropic crystals persisted, especially in the deeper portions of the intima. The superficial layers of the lesions finally consisted chiefly of fibrous connective tissue with abundant collagen and elastic fibrils.

Regression of cholesterol-induced arteriosclerosis in the dog was investigated by Bevans and others.[10] They found that aortic lesions produced by the feeding of thiouracil plus cholesterol diminished in number and severity during the period when the animals were returned to a stock diet.

There is evidence that arteriosclerotic lesions in man may regress. So-called lipid spots on the aortic intima increase in frequency during the first few decades of life but tend to disappear thereafter, and it is believed that these lesions are primarily lipid in origin. While these earlier lesions probably regress completely, other advancing arteriosclerotic lesions may occur in the same locations in middle and old age.[11] In an earlier study of lipid infiltration of human aortas, Schirmer [12] suggested that atheromatous lesions might heal by reconstitution of intimal connective tissue. Leary [13] has stated that atheromatous lesions need not advance, and, indeed, may regress, after the disappearance of cholesterol from the lesion. This regressive change was believed to occur in the ascending aorta, even in old age. According to Wilens,[14] even advanced arteriosclerotic lesions of the aortas of older persons may regress.

Horlick and Katz [15] demonstrated regression of arteriosclerosis in cholesterol-fed chicks. Within three weeks after cessation of feeding of cholesterol little or no gross evidence of arteriosclerosis remained in the aorta, a finding that suggested a complete remission. Rodbard and co-workers [16] recently presented data showing that the cholesterol-induced arteriosclerotic lesions of the chick aorta, which were visible grossly, might resolve rapidly. Peterson and Hirst [17] also studied the relation of diet and cholesterol to atheroma formation in chickens and concluded that reversibility of cholesterol-induced aortic lesions was related to the total amount of cholesterol ingested and to the duration of the cholesterol feeding.

Thus, in rabbits, dogs, birds, and, possibly, man, regression of naturally occurring and experimentally induced arteriosclerosis does occur.

2. *Regression of Arteriosclerosis in the Present Study.*—The apparent persistence or lack of significant regression of the experimentally induced abdominal lesion may be due, in part, to the continuing development of the naturally occurring lesion. It has been previously pointed out that the lesions induced in birds by the feeding of cholesterol differed from those induced by the injection of diethylstilbestrol in that the proportion of cholesterol deposited in the vascular wall was apparently greater in the birds fed cholesterol.[1] When cholesterol was deposited in the arterial wall and, particularly, after it had formed large crystals, it seemed to be removed less readily than did other lipid substances.

The factors probably accounting for the persistence of lipid and cholesterol in the abdominal intimal plaques of both diethylstilbestrol-injected and cholesterol-fed birds following cessation of the experimental procedures are (1) the relative localization of cholesterol and other lipid substances in the central and deeper portions of the plaques and (2) the dense peripheral fibrosis about these lipid-containing areas.

It is evident that the degenerative changes occurring in the experimental lesions, induced by either diethylstilbestrol or cholesterol, are fundamentally similar and are characterized by loss of lipid, condensation of intercellular stroma, formation of cholesterol crystals surrounded by foreign-body giant cells, and deposition of calcium. The degenerative changes of the regressing lesion, however, are more pronounced in the cholesterol-fed birds.

Previous studies of regression of arteriosclerosis in the bird were made on younger animals fed cholesterol.[15] Much greater regression was observed in those birds than in the older birds used in the present study. In our birds, regression of experimentally induced lesions, during the 8 to 12 months after removal of the experimental stimulus, was not particularly impressive. However, the thoracic lesions in both the diethylstilbestrol-injected and the cholesterol-fed birds did show some regression, mainly indicated by a decrease in the amounts of intimal and medial lipid. For purposes of studying the disappearance of lipid from a vascular wall, the thoracic aorta is, therefore, the tissue of choice.

There was little evidence of regression of the experimentally induced lesions in the abdominal aorta. It is of interest to note that the abdominal lesion in the bird closely resembles, in its pathogenesis and histologic appearances, the arteriosclerotic lesion of muscular arteries of man. Both lesions originate at sites of injury of the internal elastic membrane. In both, accumulations of acid mucopolysaccharide are replaced by fibroblastic proliferation and collagen, and these, in turn, are followed by lipid and cholesterol deposits, appearing first in the deeper layers of the thickened intima. Since it is probable that these lesions in the bird and in man are comparable, it would appear that various regimens designed to lower plasma lipids and cholesterol in man would result in little, if any, regression, once severe arteriosclerotic lesions have been established.

SUMMARY

The following two phases of the arteriosclerotic process of the bird were studied: (*a*) the developmental aspects of both the naturally occurring and the cholesterol- and diethylstilbestrol-induced lesions of the thoracic and abdominal aorta and (*b*) the regressive changes occurring in the established, experimentally induced lesions when the atherogenic stimulus of lipemia had been removed.

In the control birds the thoracic aortic lesion resulted from lipid infiltration, first in the media and later in the intima. Proliferation of intimal cells led to intimal thickening and formation of plaques.

The initial abdominal aortic lesion consisted of fragmentation of the internal elastic lamina, followed by deposition of acid mucopolysaccharide. Intimal fibroblastic proliferation associated with further mucoid deposition resulted in formation of large intimal plaques. Lipids appeared to play no part in the pathogenesis of the early abdominal lesion, although lipids did appear later in the deeper portions of larger intimal plaques.

The pathogenesis of the thoracic and abdominal lesions of birds injected with diethylstilbestrol or fed cholesterol is similar to that of the naturally occurring disease, but the experimentally induced disease is severer because of greater degrees of lipid infiltration with its complicating features.

Regressive changes in the aortic lesions were observed when lipemia, previously induced either by diethylstilbestrol injection or by cholesterol feeding, disappeared.

Regression of the thoracic lesion was characterized by a decrease in size and number of intimal plaques, by decreased amounts of

infiltrating lipids (including refractile substances), and by increasing amounts of intercellular fibrillary material.

There was little evidence of regression of the experimentally induced lesions in abdominal aortas, although deposition of crystalline cholesterol, increased amounts of condensed connective tissue stroma, and calcific deposits were observed in the postlipemic period. This late lesion of the abdominal aorta closely resembled late arteriosclerotic lesions of muscular arteries of man.

The California Poultry Supply Co., Los Angeles, supplied the Capette Pellets (diethylstilbestrol) used in this study, and Mr. William R. Gaffey, Research Statistician in the School of Public Health, did the statistical analysis of the blood lipid data.

REFERENCES

1. Chaikoff, I. L.; Lindsay, S.; Lorenz, F. W., and Entenman, C.: Production of Atheromatosis in the Aorta of the Bird by the Administration of Diethylstilbestrol, J. Exper. Med. **88**:373, 1948.

2. Sperry, W. M., and Brand, F. C.: Colorimetric Determination of Cholesterol, J. Biol. Chem. **150**:315, 1943.

3. Chaikoff, I. L., and Kaplan, A.: Blood Lipids in Completely Depancreatized Dogs Maintained with Insulin, J. Biol. Chem. **106**:267, 1934.

4. Moon, H. D., and Rinehart, J. F.: Histogenesis of Coronary Arteriosclerosis, Circulation **6**:481, 1952.

5. Taylor, H. E.: Role of Mucopolysaccharides in the Pathogenesis of Intimal Fibrosis and Atherosclerosis of the Human Aorta, Am. J. Path. **29**:871, 1953.

6. Lindsay, S.; Chaikoff, I. L., and Gilmore, J. W.: Arteriosclerosis in the Dog: I. Spontaneous Lesions in the Aorta and the Coronary Arteries, A. M. A. Arch. Path. **53**:281, 1952.

7. Lindsay, S.; Feinberg, H.; Chaikoff, I. L.; Entenman, C., and Reichert, F. L.: Arteriosclerosis in the Dog: II. Aortic, Cardiac, and Other Vascular Lesions in Thyroidectomized-Hypophysectomized Dogs, A. M. A. Arch. Path. **54**:573, 1952.

8. Anitschkow, N.: Experimental Arteriosclerosis in Animals, in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, pp. 271-322.

9. Duff, G. L.: Experimental Cholesterol Arteriosclerosis and Its Relationship to Human Arteriosclerosis, Arch. Path. **20**:81, 259, 1935.

10. Bevans, M.; Davidson, J. D., and Kendall, F. E.: Regression of Lesions in Canine Arteriosclerosis, A. M. A. Arch. Path. **51**:288, 1951.

11. Hueper, W. C.: Arteriosclerosis: A General Review, Arch. Path. **39**:187, 1945.

12. Schirmer, H. K.: Beitrag zur Histologie und Bedeutung der Virchowschen "fettigen Usur" der Aorta, Arch. Path. Anat. **230**:191, 1921.

13. Leary, T.: Cholesterol Lysis in Atheroma, Arch. Path. **37**:16, 1944.

14. Wilens, S. L.: Resorption of Arterial Atheromatous Deposits in Wasting Disease, Am. J. Path. **23**:793, 1947.

15. Horlick, L., and Katz, L. N.: Retrogression of Atherosclerotic Lesions on Cessation of Cholesterol Feeding in the Chick, J. Lab. & Clin. Med. **34**:1427, 1949.

16. Rodbard, S.; Bolene-Williams, C.; Pick, R., and Katz, L. N.: Beneficial Effects of Intermittent Dietary Regimens on the Tendency to Atherosclerosis in Chicks, to be published.

17. Peterson, J. E., and Hirst, A. E., Jr.: Studies on the Relation of Diet, Cholesterol and Atheroma in Chickens, Circulation **3**:116, 1951.

PATHOLOGY OF CHOLINE DEFICIENCY IN THE MOUSE

Observations with Special Reference to Liver

GEORGE F. BUCKLEY, M.D., Stouffville, Ont. Canada
and
W. STANLEY HARTROFT, M.D., Ph.D., St. Louis

SINCE THE discovery of the lipotropic action of dietary choline and its precursors, betaine, methionine, and methionine-containing protein, beginning with the original observations of Best and Huntsman in 1932,[1] many reports have appeared in the literature concerning the pathology of choline deficiency in the rat,* dog,† chicken,‡ rabbit,§ guinea pig,‖ monkey,[15] and, probably, man.¶ The mouse was one of the first animals in which a fatty liver due to low-choline diet was demonstrated. In 1932, Best, Huntsman, and Solandt [18] reported that mice fed diets low in choline rapidly developed fatty livers. These initial observations on the mouse were soon confirmed by a number of subsequent investigations,[19] but they were all concerned with the biochemical aspects of the hepatic changes. Wilson investigated neoplastic changes in livers of choline-deficient mice.# In his experiments nodular cirrhosis did not develop in the livers of mice maintained on a 4% casein diet for long periods of time or fed a semisynthetic diet mixed with an equal amount of bentonite, a cation-exchange silicate which prevents absorption of choline. Wilson discovered hepatomas in all but one of 12 mice fed the bentonite-diet mixture for 200 days. He described the tumors as resembling masses of liver parenchyma without normal lobular pattern and without portal canals. Highman and Daft noted that cirrhosis developed in C3H mice fed low-protein, low-choline diets [24] but did not describe the lesions extensively.

The present report is concerned with the detailed study of the series of pathologic changes observed in the livers of 51 mice fed a low-choline diet for periods up to 172 days, along with studies of a comparable number of controls fed the basal diet supplemented with adequate amounts of choline. The observations of Wilson have been confirmed and extended. The pathology of choline deficiency in the livers of mice will be compared to that in rats.

MATERIAL AND METHODS

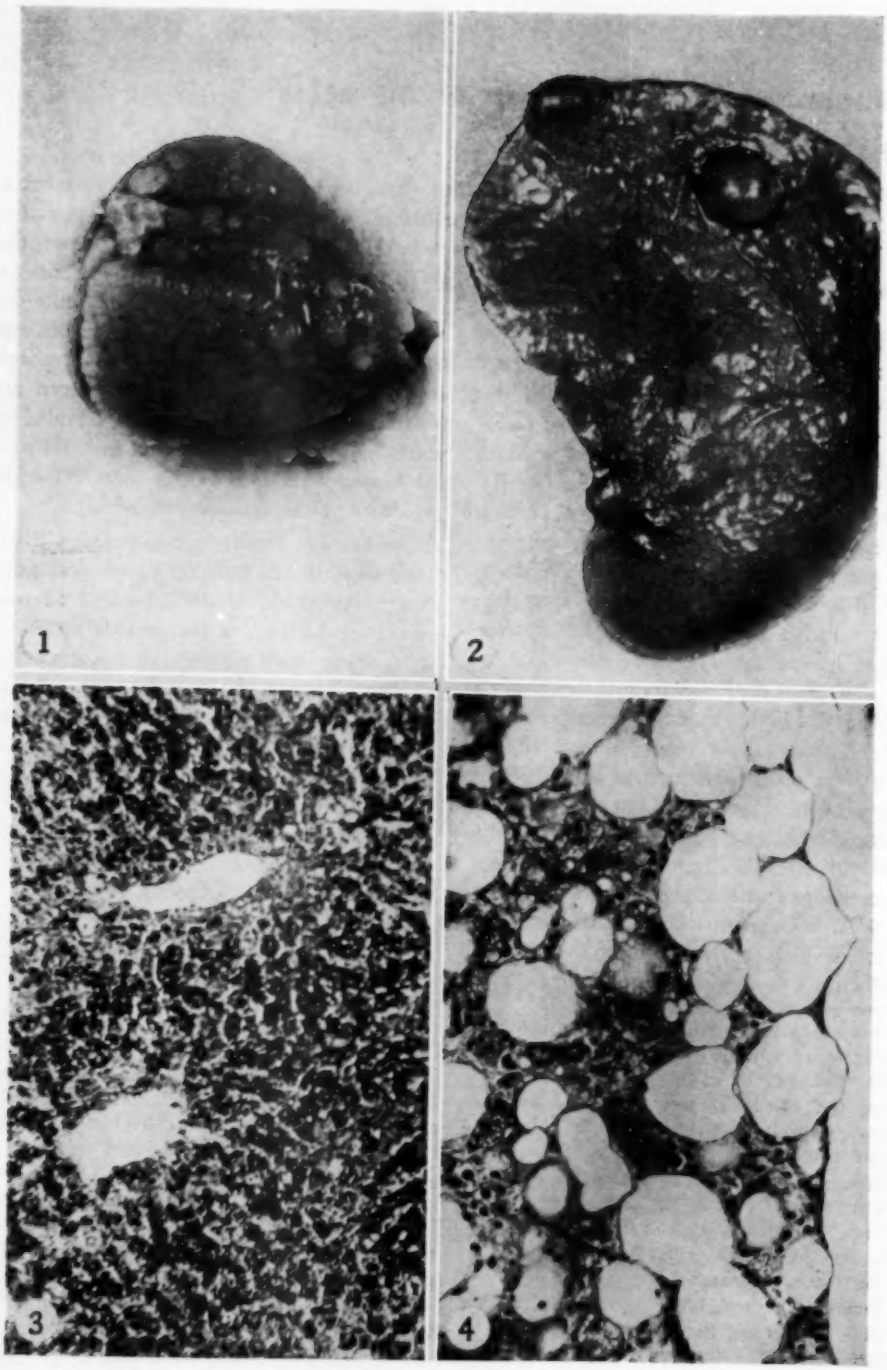
One hundred ten weanling male mice, 3 weeks old, of the Carworth Farm strain were studied in two experiments. In the first of these, 90 animals were employed, 45 being fed a basal choline-deficient diet and 45 this diet supplemented with 0.5% choline chloride. During the first two and one-half months of the experiment the animals were kept five to a cage; then, for the remainder of the period, all mice were transferred to individual cages. Mice from both groups, experimental and control, were serially killed at intervals of 3, 8, 11, 13, 15, 20, and 25 weeks. Animals were killed under ether-inhalation anesthesia and representative blocks of tissue taken from heart, aorta, liver, pancreas, kidney, spleen, testes, and most other viscera. Blocks of tissue were fixed by immersion in Bouin's solution and in formol-calcium solution. Bouin-fixed tissues were dehydrated in isopropyl alcohol using a Technicon, infiltrated and embedded with wax, and sectioned at 5μ. Hematoxylin and eosin stain on paraffin sections was done routinely, and, in addi-

From the laboratories of the Banting and Best Department of Medical Research, University of Toronto, Canada.

Supported in part by grants from the National Research Council of Canada and the Nutrition Foundation Inc., U. S. A.

Portions of this paper have been presented by one of the authors (W. S. H.) at the International Congress of Clinical Pathologists, Washington, D. C., Sept. 6-12, 1954.

* References 2 through 5.
† References 6 through 8.
‡ References 9 and 10.
§ References 11 and 12.
‖ References 13 and 14.
¶ References 16 and 17.
# References 20 through 23.



(See legends on opposite page)

tion, on selected tissues special stains were carried out to demonstrate elastic tissue, connective tissue, ceroid, hemosiderin, and calcium. Formalin-fixed tissues (hearts, livers, kidneys, and, in some instances, aortas) were frozen, sectioned at 5μ, and stained by Wilson's modification of Lillie's isopropyl alcohol (Isopropanol) supersaturated oil red O technique.[25]

Stainable fat in abundance was found in the livers of mice in the first group killed at the end of only three weeks of choline deficiency. Therefore, in a second experiment to study earlier stages of the condition, 10 weanling mice were fed the choline-deficient diet and 10 were fed this basal diet supplemented with 0.5% choline chloride. Two animals from each group were killed after intervals of 2, 4, 8, 16, and 24 days on their respective dietary regimens. Histologic techniques applied to tissues obtained from these animals were the same as those employed in the first experiment.

The percentage composition of the basal hypolipotropic diet was as follows: peanut meal 6,* soya protein 6,† PDW-vitamin mixture 1,‡ LP salts 3,§ Cellu flour 2, cystine 0.15, dextran 18.0, starch 18.0, sucrose 20.85, lard 25.0, cod liver oil concentrate 0.010,‖ alpha-tocopherol acetate 0.010. The dietary ingredients were mixed in a Hobart power food mixer and the rations were stored in tightly covered tinned cans in a refrigerator at about 4 C. The diet was placed in feeding tins made from ointment containers, which were specially devised to minimize spilling. The amount of food consumed by the animals was not recorded. Some of the animals practiced coprophagia and those that did this consistently developed only mild signs of lipotropic deficiency. Individual cages were devised from empty coffee or tobacco tins, four inches high and four inches in diameter, with holes punched in the removable tops. Sawdust was sprinkled on the bottom and a one-quarter inch mesh, galvanized wire screen floor, raised one inch from the bottom of the tin, was inserted. Tap water was available to the animals at all times.

* Solvent process; peanut meal extracted with hot ethanol (50%, 70%, and 95%, respectively).

† Glidden's "alpha protein."

‡ The PDW-vitamin mixture consisted of thiamine hydrochloride, 500 mg.; riboflavin, 250 mg.; pyridoxine hydrochloride, 200 mg.; calcium pantothenate, 1 gm.; nicotinic acid, 1 gm.; folic acid, 50 mg.; biotin, 30 mg.; menadione (2-methyl-1, 4-naphthoquinone), 100 mg.; *p*-aminobenzoic acid, 10 gm.; inositol, 50 gm.; finely powdered sucrose (100 mesh), to 1,000 gm.

EXPLANATION OF FIGURES 1, 2, 3, 4

Fig. 1.—Surface of the liver of a mouse that had been fed the low-choline diet for 60 days. The liver is greatly swollen by contained fat, rendering the lobular pattern very prominent. A few large subcapsular fatty cysts may be seen; × 8.

Fig. 2.—Two large nodules are seen on the surface of the liver of a mouse that had been fed the low-choline diet for 150 days. Microscopically, these nodules proved to be hepatomas (Fig. 12); × 4.

Fig. 3.—Stainable fat is present in small droplet form within nearly every parenchymal cell in this liver of a mouse that had been fed the low-choline diet for only two days. The cells immediately surrounding the branch of the portal vein in the upper portion of the field contain a little less fat than the remainder. Frozen section stained with oil red O; × 100.

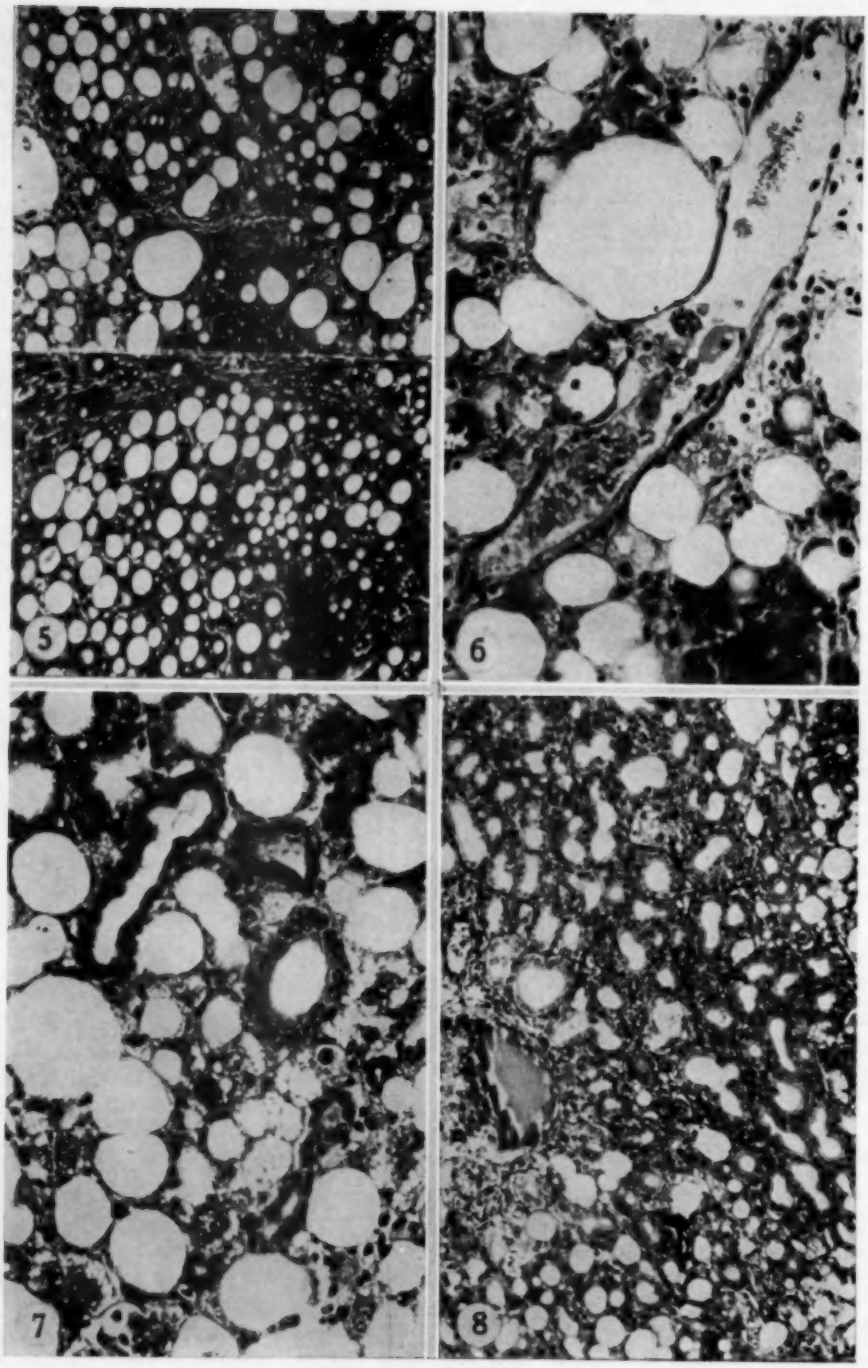
Fig. 4.—Large fatty cysts have formed throughout this liver of a mouse that had been fed the low-choline diet for 50 days. Subcapsular cysts are bulging outward on the surface at the right. At the lower right, two cysts are fusing into a single larger one. Paraffin section stained with hematoxylin and eosin; × 200.

RESULTS

The gross appearances of the livers in the choline-deficient mice were striking (Figs. 1 and 2). After three or four weeks of choline deficiency the livers had enlarged to two or three times their normal volume and extended almost to the lower limit of the abdominal cavity. But "hobnail" livers were never encountered, even in mice that had been maintained on low-choline diets for six months. Although these livers appeared finely nodular, they felt no firmer than usual and the surface irregularity was found microscopically to be due to fatty cysts that bulged beneath the capsule (below). The pattern of the lobules (Fig. 1) was prominent because each was distended with fat. In some

§ The salt mixture (Lucas and Patterson) is made from salts commercially available in finely powdered form and supplying amounts of minerals believed optimal for growth of rats; 1 kg. contains $CaCO_3$, 110 gm.; $CaHPO_4$, 325; K_2HPO_4, 275; $MgSO_4.3.5H_2O$, 100; NaCl, 150; ferric citrate, 30; "trace element mixture," 10 gm. The last contains, per 100 gm.: $CaCO_3$, 70; $MnSO_4.4H_2O$, 19.85; $ZnSO_4.7H_2O$, 3.50; $CuSO_4.5H_2O$, 4.00; KI, 0.05; NaF, 0.05; $Al_2(SO_4)_3.K_2SO_4.24H_2O$, 0.40; $CoCl_2.6H_2O$, 0.05; $Na_2SiO_3.9H_2O$, 2.00, and $NaAsO_2$, 0.10.

‖ The cod liver oil concentrate was obtained from Ayerst, McKenna, & Harrison, Ltd., Montreal, Canada. It contains per gram 200,000 international units of vitamin A and 50,000 international units of vitamin D.



(See legends on opposite page)

late stages isolated nodules were observed scattered apparently at random on the surface, the largest measuring 5 to 6 mm. in diameter.

Appreciable amounts of stainable fat had accumulated within nearly every parenchymal cell in the livers of animals killed after they had consumed the choline-deficient diet for periods as brief as two days (Fig. 3). As a result, it was difficult to determine any characteristic lobular distribution of the lipid which might be evident in even earlier stages, although in a few areas of some of the sections there was less stainable fat in liver cells immediately adjacent to portal triads. In the rat, dietary choline deficiency regularly results in a nonportal distribution of the stainable fat.¶ A similar type of distribution in the choline-deficient mice was not clearly demonstrated, and with this object even briefer periods of choline deficiency than two days are now being studied.# The stainable fat present at the end of two days of choline deficiency was in small cytoplasmic droplets that surrounded, but did not displace, the nuclei. Most liver cells in section contained from two to eight of these droplets.

¶ References 2 through 5.

Experiments with this objective are now being carried out in this laboratory by Mr. J. Blumenstein and Mr. Henry Best. Their results will be reported separately elsewhere.

EXPLANATION OF FIGURES 5, 6, 7, 8

Fig. 5.—The upper half of the picture shows a low-power view of the liver of a choline-deficient mouse, and the lower half, taken at the same magnification, represents the liver of a choline-deficient rat. Note the greater size of fatty cysts in the mouse liver. Paraffin sections stained with hematoxylin and eosin; × 100.

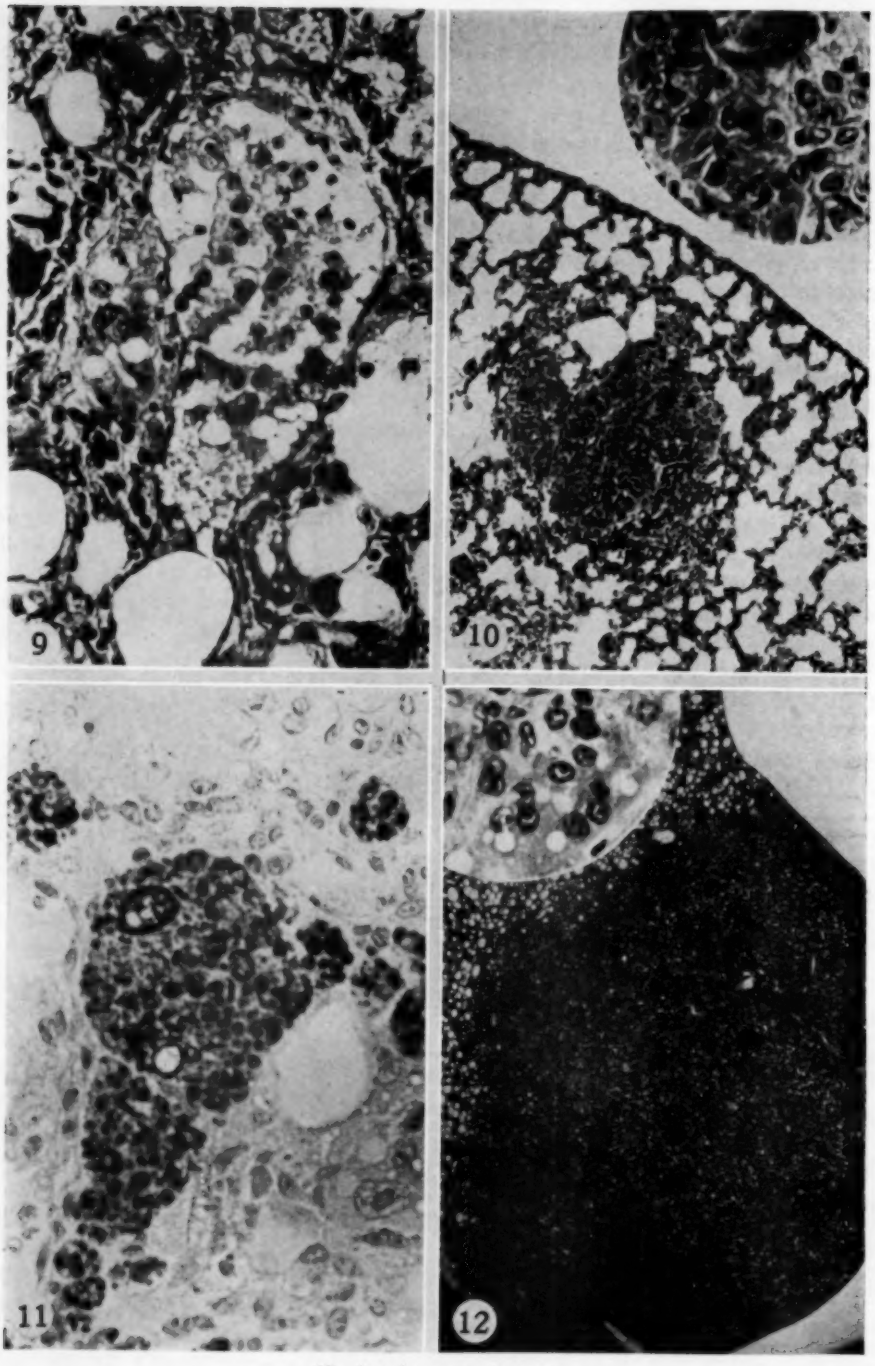
Fig. 6.—A fatty cyst (upper center) is bulging into the lumen of the radicle of the hepatic vein, cut tangentially, in this paraffin section of the liver of a choline-deficient mouse. Trichrome stain; × 200.

Fig. 7.—A normal bile duct is shown in the right center portion of the field illustrated, from a paraffin section of liver of a choline-deficient mouse. Above the bile duct and also to the left are two newly formed ducts. Note that the latter stain more darkly, and the cells are small. Trichrome stain; × 200.

Fig. 8.—Extensive bile duct hyperplasia, presenting an adenomatous appearance, is shown in this low-power view of a paraffin section of a choline-deficient mouse liver. Trichrome stain; × 50.

In the livers of the mice killed after 4, 8, 16, and 24 days of choline deficiency, stainable fat in the cytoplasm of the parenchymal cells rapidly increased. Intracellular lipid droplets fused together to form large spherules that occupied nearly all the cytoplasm and displaced the nuclei to one side. Fatty cysts (originally termed lipodiastemata [5]) were present in livers of all animals that were killed after more than one month of choline deficiency (Fig. 4), and cysts were noted in the liver of one mouse that had been fed the low-choline diet for only eight days. The manner in which fatty cysts are formed and the stages in their development in rats have been previously described.[26] Formation of fatty cysts in choline-deficient mice follows exactly the same pattern as in the rat; accumulation of increasing amounts of fat in adjacent liver cells compresses their contiguous limiting membranes into an optically single tenuous septum that separates the two masses of intracellular lipid. Still further accumulation of fat within these cells may stretch the septum until it ruptures, thus allowing the two pools of lipid (now separated by the septum no longer, since it has been torn) to coalesce. The ruptured cells are conjoined by the fragments of the torn septum formed originally by compression of their limiting membranes, and, in effect, thereby constitute the walls of small cysts comprised of two or more such cells. In the choline-deficient rat, fatty cysts rarely attained diameters of more than 100μ in paraffin sections (compared to diameters of 12 to 14μ for single liver cells in similar sections of control rats' livers). Cysts in the choline-deficient mice eventually became much larger than any ever encountered in the rats, with diameters in some sections of 150μ or more (Fig. 5).

These large cysts in the mouse livers sometimes presented unusual features. Frequently they were observed immediately beneath the capsule of Glisson, bulging on the surface of the organ (Fig. 4). These bulges undoubtedly were smaller in the sections than they would have been during the life of the animal, owing to shrinkage of the



(See legends on opposite page)

tissues during fixation and dehydration. In some instances the tenuous cyst membrane separating the fat from the peritoneal cavity was ruptured in the section, but this appearance was undoubtedly, in many instances, due to artifact, because inflammatory reactions in these regions were not encountered in the sections. The largest of the subcapsular cysts were visible on gross examination as small, yellow, glistening excrescences.

In the substance of the liver, fatty cysts sometimes compressed and encroached upon blood vessels. This situation was observed most frequently around radicles of the hepatic vein (Fig. 6), where the cysts bulged against the vessel wall, pushing it inward. At these points the wall of the cyst and the lining of the vessel together formed only a tenuous septum, so thin that even under the highest magnifications of the light microscope this membrane, separating the cyst contents (fat) from the blood within the vessel, could not be resolved into more than one structure.

Fibrosis in choline-deficient mice livers in these experiments never attained appreciable proportions. Cysts attained relatively enormous dimensions (Fig. 5), but ruptured cysts were not often encountered (as is the case in livers of choline-deficient rats). Between intact cysts, strands of blue-staining, coarse reticulin made their appearance in livers of mice maintained on the low-choline diets for periods of two and one-half months or more. Together, these intercystic reticulin strands give a picture of a minimal degree of diffusely distributed hepatic fibrosis. In an occasional animal, it was apparent that more condensation of reticulin had occurred in nonportal regions than in others, but well-defined focal lobular patterns of the lesions were rarely encountered; whereas, in the rat, hepatic fibrosis induced by choline deficiency is almost always delimited to nonportal regions at a comparable stage.

Lesions in Choline-Deficient and Control Mice

No. of Mice	Choline Supplement	Trace of Liver Fat	Abundant Liver Fat	Hepatic Fibrosis	Hepatic Neoplasia	Fatty Degeneration, Kidney	Calcification in Renal Arteries	Fat and Necrosis in Myocardium	Fat in Coronary Arteries
51	0	0	51	27	11	21	6	10	3
52	+	28	0	0	0	1	0	3	1

After even comparatively brief periods of choline deficiency, hyperplasia of bile ducts became a prominent feature in all sections of the mouse livers. The earliest evidence of this change was observed in one animal that had been killed after 24 days on the low-choline diet. Bile duct hyperplasia was observed to develop earliest in the midportions of liver lobes near major divisions of the biliary tree (Fig. 7). Newly formed ducts were lined by orderly rows of benign-appearing epithelial cells. Mitotic figures were frequently encountered, however, and it was evident that increase of bile ducts in the sections was a result of epithelial hyperplasia. New ducts formed areas which became large enough to occupy entire low-power fields (Fig. 8), giving an appearance suggestive of

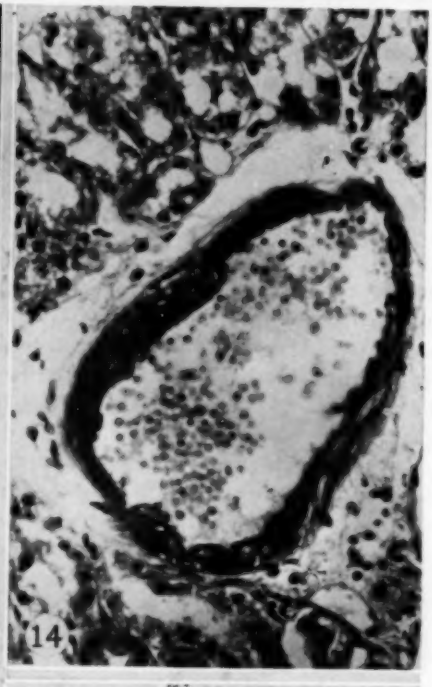
EXPLANATION OF FIGURES 9, 10, 11, 12

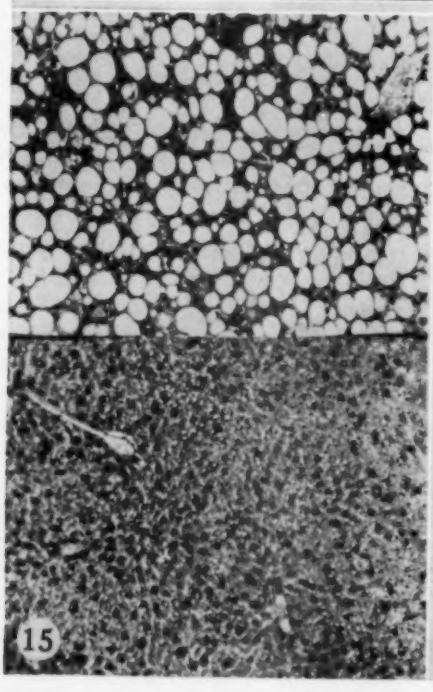
Fig. 9.—A nest of proliferating parenchymal cells is present at the left between the adventitial sheath and intimal lining of a radicle of a hepatic vein in the liver of a choline-deficient mouse. Ceroid deposits (not visible here) and fat vacuoles occupy the cytoplasm of the cells. Paraffin section with trichrome stain; × 400.

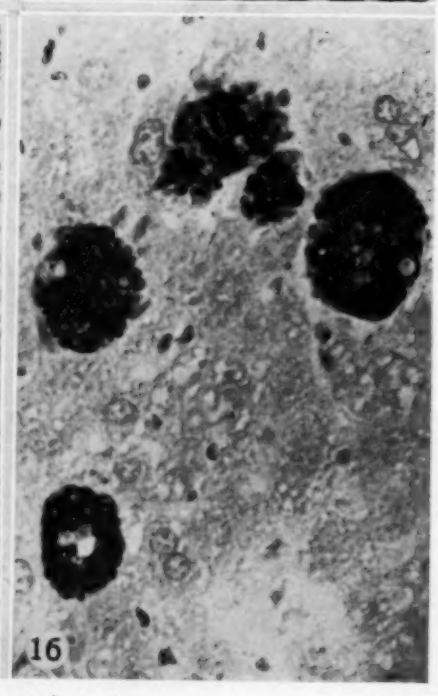
Fig. 10.—A small nodule of abnormal cells resembling those in bile ducts is present beneath the surface of the lung of a choline-deficient mouse. The inset shows an attempt at formation of ducts. Paraffin section with trichrome stain; × 100 and × 500.

Fig. 11.—A clump of ceroid pigment is shown occupying very swollen parenchymal cells, the nucleus of one being visible at the left edge of a clump. The granular nature of the ceroid can be seen. Paraffin section stained with oil red O; × 400.

Fig. 12.—The low-power view shows a nodule of the type illustrated grossly in figure 2. Fat accumulation does not develop to more than minimal degree in these nodules. The inset shows the nature of the cells which bear some resemblance to those of liver parenchyma. Paraffin section stained with hematoxylin and eosin; × 20 and × 500.







(See legends on opposite page)

adenomatous change. Nothing in their histology or cytology suggested malignancy, but in animals fed the low-choline diet for more than three months invasion of the walls of radicles of the hepatic veins by hyperplastic duct cells was encountered (Fig. 9). They formed small nodules or sheets between the adventitial sheath and the intimal lining of the vessels. Frequently these nests of proliferating cells were separated from the lumen of the vessel by only its endothelial lining. Sometimes the cytoplasm of these cells contained fat vacuoles and deposits of ceroid (Fig. 9). In the lungs of one mouse a small nodule was found near the surface of one lobe. It proved to be metastatic tumor of bile duct origin (Fig. 10). Duct hyperplasia with adenomatous change was observed in 11 of the 51 choline-deficient animals (Table).

In addition to the areas of adenomatous hyperplasia of bile ducts, regions of parenchymal cell hyperplasia were encountered (Fig. 12) that sometimes formed nodules of sufficient size that they were apparent on gross examination (Fig. 2). Nuclei of the cells forming the nodules resembled nuclei of normal liver cells with regard to nuclear membranes, size of nucleoli, and chromatin network. Cytoplasm of these cells was much less abundant than that of normal liver cells, so that nuclear-cytoplasmic ratios in the cells of the nodules were abnormally high. Lobular organization and cord formation were ill-defined in the nodules, and rarely did they contain appreciable amounts of stainable fat. All these features are characteristic of hepatomas, and, indeed, the appearance of the liver-cell nodules resembled that of transplantable liver tumors of mice, as described by Maver and co-workers.[27]

Ceroid [4] deposits formed a prominent feature in nearly all the sections of the choline-deficient mice livers. The longer the animals remained on the diet the greater the abundance of the deposits. The distribution of the ceroid pigment was clearly nonportal, being found most frequently immediately adjacent to large radicles of the hepatic vein, often in the form of perivascular cuffs. The deposits were granular in appearance, so that their resemblance to clumps of distorted red blood cells was readily apparent (Fig. 11). The possible significance of these observations will be discussed. Ceroid deposits were also found in the lungs of many of the choline-deficient mice killed in the latter half of the experimental period.

Significant abnormalities of organs other than the liver were not detected on gross examination at autopsy. But microscopically in nearly half of the choline-deficient mice (21 out of 51) renal tubules contained abundant deposits of stainable fat (Table). Lipid drops frequently filled the cytoplasm of epithelial cells forming the walls of proximal convoluted tubules. In most advanced instances cells of proximal convoluted portions of every lobule were fatty (Fig. 13), but these deposits of lipid were not associated with any other abnormalities of the tubules, such as the necrosis or atrophy so frequently encountered in kidneys of choline-deficient rats. Deposits of lipid in renal tubules could not be demonstrated in any animals killed before the 20th day of the experiment. Only the kidney of 1 of 52 choline-supplemented mice contained abnormal fat.

Hyaline degeneration and calcification of subintimal and medial layers of renal vessels were noted (Fig. 14) in 6 of 51 choline-deficient mice; this lesion was not encoun-

EXPLANATION OF FIGURES 13, 14, 15, 16

Fig. 13.—The proximal convoluted tubules in the cortex of this kidney from a choline-deficient mouse appear black because they contain masses of stainable fat. Frozen section stained with oil red O; × 20.

Fig. 14.—A renal artery from a choline-deficient mouse is shown. The vessel has not contracted. Hyalinization and calcification of intima and media have occurred at the left. Paraffin section with hematoxylin and eosin stain; × 150.

Fig. 15.—The upper portion shows the degree of fat accumulation in the liver of a mouse that had consumed the low-choline diet for 28 days. The lower portion is from the liver of a mouse that was fed the low-choline diet for a similar period and then treated with choline for 125 days. Paraffin sections with hematoxylin and eosin stains; × 80.

Fig. 16.—Higher magnification of a section of the liver illustrated in the lower portion of the preceding figure. Masses of ceroid pigment are still present in this liver and exceed, in quantity, the amount encountered in any mice killed after less than 30 days of choline deficiency (see text). Oil red O stain on paraffin section; × 300.

tered in the controls. Affected vessels in section are distended in a manner suggesting that the usual contraction seen in fixed material had not occurred. Stainable fat was not found in any of these lesions. In their early stages homogeneous eosinophilic material was deposited immediately beneath the intima and encroached, in some instances, upon the media. Only small deposits of calcium salts were present, even in more advanced stages, and took the form of fine granular clumps chiefly in the media. Neither in their pathogenesis nor in their final form did these lesions resemble those previously encountered in choline-deficient rats.* Fatty degeneration in coronary arteries and cardiac muscle was encountered more frequently in the choline-deficient group (Table) than in the choline-supplemented mice, but it was not confined to the former and its significance may be questioned.

One animal in the choline-deficient group was maintained on the basal dietary regimen for 28 days, and then for a further 125 days it was given choline (Fig. 15). Lipotropic therapy was successful in mobilizing all stainable fat from the liver cells, but striking amounts of ceroid pigment were observed on necropsy (Fig. 16) in large clumps, of the shape and size of fatty cysts. The amount of the pigment was so abundant as to suggest that perhaps it had continued to form for some time after the institution of choline therapy.

COMMENT

Fatty Cysts in Choline-Deficient Mice.—Greater amounts of stainable fat in livers of mice subjected to dietary choline deficiency accumulated more rapidly than in young rats fed similar diets for comparable periods of time. Dr. Jessie H. Ridout, in the Banting and Best Department of Medical Research, has measured biochemically the amount of liver lipid in the mice, and her results have indicated that the percentage fat (of weight of liver) exceeded anything ever encountered in the same laboratory in choline-deficient rats. Judging by the amount of liver fat, the mouse is more sensitive to choline deficiency than the rat, but this comparative rating is not true for other manifestations of the low-choline diets. Grossly nodular cirrhosis, renal damage, cardiac necrosis with fibrosis, and lesions of coronary arteries or aortas apparently can not be produced readily in the choline-deficient mouse. On the other hand, hyperplastic and neoplastic changes in the livers of the mice appeared more frequently than in the livers of the strain of rat studied in this laboratory for periods up to a year or more. Even with more susceptible strains, Copeland and Salmon,[31] who first reported that hepatomas could be induced by choline deficiency, found it took a year or more before neoplasia could be demonstrated in the animals.

Normal liver cells in mice are smaller than those in rats but, despite this, the average size of fatty cysts in the choline-deficient mice was larger than those in the choline-deficient rat. In the mice, cysts continued to enlarge instead of rupturing, as in the rat. In previous publications we have emphasized the cirrhogenic nature of fatty cysts in rats and man.[32] Livers of these choline-deficient mice provide corroborative evidence of a "negative" nature to support this concept. In the mice, fatty cysts continue to grow and enlarge, but rarely rupture, and in these livers only minimal reticulin condensation was encountered, thus confirming Wilson,† who also reported that frankly nodular cirrhosis did not develop in choline-deficient mice. Absence of cirrhosis in these animals could be explained by failure of accumulated fat to destroy liver cells by cyst rupture, as is the case in the rat. Highman and Daft,[24] who reported cirrhosis in mice, used a special strain (C3H) that appears to react in a manner more like that of the rat to choline deficiency than did the strains used by Wilson and by us.

Neoplasia.—The frequent association of cancer of the liver and cirrhosis has been well documented in the clinical literature. Most observers have assumed that neoplasia in cirrhotic choline-deficient rats' livers was secondary to the cirrhosis. In confirmation

* References 28 through 30.

† References 20 through 23.

of Wilson's findings,‡ we have also observed the development of neoplasia (with pulmonary metastasis in one case) in noncirrhotic livers of our choline-deficient mice. This confirmation would seem to establish clearly that in some strains of mice, at least, cirrhosis is not a necessary event in the pathogenesis of cancer induced by dietary deficiency. It does not imply, however, that choline deficiency directly induced neoplasia in liver cells. Neoplasia may have developed as a result of the disturbance in structure and function caused by excessive fat accumulation in the livers. In any event, dietary deficiency must now be included among other carcinogenic factors in experimental cancerology, as proposed also by Tannenbaum.[33]

Of 51 choline-supplemented mice, 22 contained an amount of stainable fat in their livers graded as 1+ and 6 were graded as 2+ (Table). In the livers of mice killed in the initial half of the experimental period, fat was most frequently periportal in distribution, but in livers of animals killed after two or more months on the control diet the fat was predominently centrolobular. The greatest amount of fat in livers of the controls was found in mice killed during the first three weeks of the experiment. The explanation for this phenomenon is not readily apparent; it is possible that excessive caloric intake by mice when first placed in individual cages might have been a factor, because they then consumed as much food as they desired without competition from other mice. Observations recently completed in this laboratory [34] have indicated that, despite adequate amounts of choline and methionine in the diet, rats that consume excessive amounts of casein diets may temporarily accumulate large amounts of liver fat, which disappear after the animals are continued on the diet for three weeks or more. Food intake in the control mice in the present experiments was not recorded. Adequate explanation for their fatty livers will have to await further and more detailed observations.

‡ References 20 through 23.

Mice are resistant to the induction of renal damage by choline deficiency. Despite appreciable amounts of abnormal lipid in the proximal convoluted tubules of many of the animals reported here, irreversible tubular damage was absent. Only Carworth Farm strain of mice was used, however, and it is possible that, if a number of other in-bred or hybrid strains of mice had been employed, renal damage might have been induced in some.

Twelve per cent of the choline-deficient mice developed hyalinization and calcification of renal arteries. These lesions were encountered more frequently in animals killed in the latter half of the experimental period, and it is possible that had all the mice been maintained on the low-choline diet for four or five months the incidence of renal vascular lesions would have been appreciably higher. Highman and Daft [24] have described similar changes in a number of vessels in C3H mice fed low-choline, low-protein diets, but they found that choline added to the drinking water of control animals did not prevent the development of the vascular changes. In the strain of mice employed in our experiments, the addition of choline to the food mixture appeared to prevent the appearance of vascular changes in control animals. The method of administering the choline supplement and variations associated with the strain of mouse appear to warrant further investigation with regard to vascular degeneration associated with low-protein diets.

Perhaps one of the most interesting results of this investigation is the demonstration that signs of a dietary deficiency in one particular strain of one species do not duplicate the lesions seen in other species (rat, guinea pig, dog, etc.). The cardinal pathologic feature of choline deficiency is the accumulation of abnormal amounts of liver fat, but its effects in the various species appear to produce divers manifestations, i. e., cirrhosis in rats, tumors in mice. These observations serve to demonstrate that undue emphasis should not be placed on detailed pathologic

criteria in comparisons of disease states in different species. Cirrhosis in choline-deficient rats is not the exact morphologic counterpart of cirrhosis in alcoholic man. The difference between the liver of the choline-deficient rat and that of the choline-deficient mouse is even greater. It would not be more justified to conclude that the etiology and pathogenesis of cirrhosis in alcoholic man and choline-deficient rat were on different bases because the lesions are not identical than it would be to assume that the etiology of liver lesions in a choline-deficient mouse and choline-deficient rat differed because their pathologic pictures varied. Only by careful studies of the pathology of dietary deficiencies in every available species of experimental animals can the investigator become familiar with the possible variations in the manifestation of the deficiency states. The accumulated data, when available, will provide information of value in assessing possible causes of disease in man. The present investigations are but one step in this direction.

SUMMARY

In male mice of the Carworth Farm strain fed choline-deficient diets for periods up to a maximum of 172 days, excessive amounts of abnormal fat were found deposited in their livers. Lipid was contained in relatively enormous fatty cysts which only infrequently ruptured. Fibrosis was minimal in any of the animals' livers, and grossly nodular cirrhosis did not develop. Adenomatous hyperplasia and hepatomas were observed in 11 of 51 choline-deficient mice. Kidneys of these animals frequently contained stainable fat deposits in proximal convoluted tubules, but other lesions were never observed in the renal parenchyma. In 12% of the deficient animals hyaline degeneration and calcification of renal arteries were encountered. Control animals, fed the same basal diet, supplemented with 0.5% choline chloride, failed to develop any significant incidence of abnormalities. Small amounts of stainable fat appeared transiently (in the initial periods of the experiment, particularly) in the livers of mice in the choline-supplemented group. Fat deposition may have been due to excessive caloric intake during that period of the experiment.

Differences between the pathology of the livers of choline-deficient mice and those of choline-deficient rats and their possible significance are discussed.

Prof. C. H. Best gave help and direction in carrying out the experiments; Dr. Jessie H. Ridout assisted in preparing the diets; Mr. William D. Wilson cut and stained the microsections, and Mrs. M. E. Lindsay prepared the manuscript.

REFERENCES

1. Best, C. H., and Huntsman, M. E.: J. Physiol. **75**:405, 1932.

2. MacLean, D. L., and Best, C. H.: Brit. J. Exper. Path. **15**:193, 1934.

3. György, P., and Goldblatt, H.: J. Exper. Med. **70**:185, 1939.

4. Lillie, R. D.; Ashburn, W. H.; Sebrell, W. H.; Daft, F. S., and Lowry, J. V.: Pub. Health Rep. **57**:502, 1942.

5. Hartroft, W. S.: Anat. Rec. **106**:61, 1950.

6. Chaikoff, I. L., and Connor, C. L.: Proc. Soc. Exper. Biol. & Med. **43**:638, 1940.

7. Chaikoff, I. L.; Connor, C. L., and Bishkind, G. R.: Am. J. Path. **14**:101, 1938.

8. Bellows, J. G., and Chinn, H.: Arch. Ophth. **30**:105, 1943.

9. Jukes, T. H.: J. Am. Vet. M. A. **102**:96, 1943.

10. Salmon, W. D., and Copeland, D. H.: Ann. New York Acad. Sc. **57**:664, 1954.

11. Rich, A. R., and Hamilton, J. D.: Bull. Johns Hopkins Hosp. **66**:185, 1940.

12. Rich, A. R., and Hamilton, J. D.: Tr. A. Am. Physicians **55**:133, 1940.

13. Casselman, W. G. B., and Williams, G. R.: Nature, London **173**:210, 1954.

14. Reid, M. E.: Fed. Proc. **13**:474, 1954.

15. Mann, G. V.; Andrus, S. B.; McNally, A., and Stare, F. J.: J. Exper. Med. **98**:195, 1953.

16. Hartroft, W. S.; Lucas, C. C., and Best, C. H.: Pathology of Choline Deficiency in Man, in The Vitamins, edited by W. H. Sebrell Jr. and R. S. Harris, New York, Academic Press, Inc., 1952.

17. Leevy, C. M.; Zinke, M. R.; White, T. J., and Gnassi, A. M.: A. M. A. Arch. Int. Med. **92**: 527, 1953.

18. Best, C. H.; Huntsman, M. E., and Solandt, O. M.: Tr. Roy. Soc. Canada **26**:175, 1932.

19. Welch, A. DeM., and Welch, M. S.: Proc. Soc. Exper. Biol. & Med. **39**:7, 1938.

20. Wilson, J. W.: J. Nat. Cancer Inst. **14**:57, 1953.

21. Wilson, J. W.: J. Nat. Cancer Inst. **14**:65, 1953.

22. Wilson, J. W.: Cancer Res. **11**:290, 1951.

23. Wilson, J. W.: Ann. New York Acad. Sc. **57**:678, 1954.

24. Highman, B., and Daft, F. S.: A. M. A. Arch. Path. **52**:221, 1951.

25. Wilson, W.: Bull. Internat. A. M. Mus. **31**: 216, 1950.

26. Hartroft, W. S., and Ridout, J. H.: Am. J. Path. **27**:951, 1951.

27. Maver, M. E.; Greco, A. E.; Løvtrup, E., and Dalton, A. J.: J. Nat. Cancer Inst. **13**:687, 1952.

28. Wilgram, G. F.; Hartroft, W. S., and Best, C. H.: Science **119**:842, 1954.

29. Buckley, G. F., and Hartroft, W. S.: J. Clin. Nutrition, to be published.

30. Hartroft, W. S.; Ridout, J. H.; Sellers, E. A., and Best, C. H.: Proc. Soc. Exper. Biol. & Med. **81**:384, 1952.

31. Copeland, D. H., and Salmon, W. D.: Am. J. Path. **22**:1059, 1946.

32. Hartroft, W. S.: A. M. A. Arch. Path. **55**: 63, 1953.

33. Tannenbaum, A.: Nutrition and Cancer, in The Physiopathology of Cancer, edited by F. Hokburger and W. H. Fishman, New York, Paul B. Hoeber, Inc., 1953.

34. Best, C. H.; Hartroft, W. S.; Lucas, C. C., and Ridout, J. H.: Brit. J. Exper. Path., to be published.

HEPATIC FIBROSIS IN CHILDREN WITH ACUTE LEUKEMIA AFTER THERAPY WITH FOLIC ACID ANTAGONISTS

JACOB COLSKY, M.D., Brooklyn
EZRA M. GREENSPAN, M.D., New York
and
THOMAS N. WARREN, M.D., Staten Island, N. Y.

AFTER Farber's initial report [1] of the development of remission in acute leukemia in children treated with aminopterin (4-aminopteroylglutamic acid), a number of investigators have amply confirmed that the anti-folic-acid compounds may induce hematological and clinical remissions in patients ill from acute leukemia.* The leukemic process ultimately becomes refractory or resistant to further treatment, even in those patients in whom excellent clinical and hematological remissions have been induced one or more times.

Unfortunately, the margin of safety with the folic acid antagonists is narrow, and undesirable side-effects may occur with doses near, or slightly in excess of, the therapeutic levels. Leucopenia, thrombopenia, anemia, painful oral mucosa ulcerations, ulcerations of the gastrointestinal tract, skin rashes, and alopecia have been noted.† Other evidence of interference with folic acid metabolism is the appearance of macrocytic red blood cells in the peripheral blood and of megaloblasts in the bone marrow.‡ Suppression of excretion of 11-oxysteroids in the urine of patients receiving amethopterin (4-amino-10-N-methylpteroylglutamic acid) or aminopterin has also been reported.§

In the course of investigating the effects of aminopterin and amethopterin in various neoplastic processes, we have treated seven children ill from acute leukemia with these compounds.‖ Five of these patients showed significant improvement in their hematological and clinical status. The hematological remissions in these patients varied from improvement in anemia, thrombopenia, and differential count in the peripheral blood to a return of the peripheral blood and marrow to an apparently normal hematological status.[8] Marked reduction in size of lymph nodes, liver, and spleen occurred one or more times in each of these patients.

Three of these five patients in remission received continuous therapy with amethopterin for periods of 9 to 12 months, while the remaining two received intermittent therapy with aminopterin or amethopterin over a period of about one year. Undesirable reactions, such as noted above, were induced at one or more times during the course of therapy in each of these patients.

No detailed pathologic studies have been reported on children with acute leukemia responsive to anti-folic-acid therapy. Rice men-

From the Clinical Research Unit of the National Cancer Institute, U. S. Public Health Service, Department of Health, Education and Welfare, Baltimore.

A preliminary report of this paper was presented at a symposium on "The Chemistry and Biology of the Pteridines" at the Ciba Foundation, London, England, in March, 1954.

Present address: Department of Medical Services of the Maimonides Hospital of Brooklyn and the Department of Medicine of the State University of New York College of Medicine at New York City, Brooklyn (Dr. Colsky); Department of Medicine, Mount Sinai Hospital (Dr. Greenspan); U. S. Public Health Service Hospital, (Dr. Warren).

* References 2 through 7.

† References 1, 2, and 5 through 8.

‡ References 5, 7, and 9.

§ References 10 and 11.

‖ The aminopterin and amethopterin used in this study were supplied by Dr. J. M. Ruegsegger, of Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

tioned the devlopment of "marked scarring of the liver similar to that seen in cirrhosis" in four of several children with leukemia treated with folic acid antagonists.[7] Farber has also observed the development of hepatic fibrosis in leukemic patients treated with these compounds. ¶

In the five patients in whom we observed remissions of the leukemic process, after folic acid antagonist therapy, we noted the development of clinical and laboratory findings consistent with the diagnosis of hepatic cirrhosis. Postmortem examinations were performed in three of these five patients. In each autopsied case there was histological evidence of hepatic fibrosis. This report summarizes the clinical findings in each of these five cases and provides a description of the pathological changes found in the liver of the three patients who came to autopsy.

Summary of Drug Dosage, Clinical Findings, and Blood Chemical Values in Patients with Hepatic Fibrosis

Patient	Treatment	Daily Dose, Mg.	Treatment Duration, Days	Total Dose, Mg.	Induced Remission	Hepatomegaly with Induced Regressions	Ascites	Abdominal Collateral Venous Channels	Blood Chemistry Examinations on Final Hospital Admission: Globulin, Gm. %	Albumin, Gm. %	Thymol Turbidity, Units	Zinc Sulfate Turbidity, Units
D. W.	Amethopterin	5-10	188	865	+	+	+	+	3.4	2.8	14.7	22.8
J. P.	Amethopterin	5-7.5	275	1057	+	+	+	+	3.2	2.5	14.0	18.3
F. N.	Amethopterin	2.5-5	240	237	+	+	+	+	3.5	2.3	37	55
L. J.	Aminopterin	1-2	420	Unknown	+	+	+	+	3.0	4.7	5	8.9
.....	Amethopterin	2.5-5	...	525	..	..	..	..	...	...	....	....
M. K.	Amethopterin	2.5-5	211	675	+	+	+	+	3.3	3.6	6.9	18

REPORT OF CASES

CASE 1.—The patient was a 5-year-old boy with acute lymphatic leukemia, admitted to the hospital on Feb. 6, 1951. The liver was enlarged 5 cm. below the costal margin, and the spleen was palpable 2 cm. below the costal margin.

Hematological examination and sternal marrow smear confirmed the diagnosis of acute leukemia.

After a total dose of 33 mg. of amethopterin over a six-day period, the lever, spleen, and lymph nodes decreased rapidly in size and symptomatic and objective improvement was observed.

Intermittent therapy with amethopterin was continued over the following four-month period. The liver, lymph nodes, and spleen enlarged thrice during this period but regressed in size each time after amethopterin therapy. Recurrence of enlarged liver and spleen and fever necessitated readmission on June 11. At this time the liver was 4 cm. below the costal margin, the spleen was 1 cm. below the costal margin, and pancytopenia was present.

After treatment with 20 mg. of amethopterin over an eight-day period, the patient improved clinically and hematologically. Corticotropin in a total dose of 1,300 mg. was then administered over the following 28-day period, with further clinical improvement.

The patient was discharged from the hospital but was readmitted on Aug. 25 because of return of lymphadenopathy, anemia, and fever. Treatment with 150 mg. of amethopterin over the following 28-day period caused a decrease in the size of the enlarged peripheral nodes, but reduction in liver size did not occur.

On Sept. 26 the blood bilirubin was 7 mg. per 100 cc., alkaline phosphatase 20 Bodansky units, and thymol turbidity value 10 units. The patient was admitted to the U. S. Public Health Service Hospital on Oct. 11.

Generalized lymphadenopathy was present, and dilated veins were present over the anterior wall of a markedly protuberant abdomen. The liver was firm and extended 6 cm. below the costal margin.

Laboratory Studies: Urea nitrogen 8 mg. per 100 cc.; total protein 5.8 gm. per 100 cc., with albumin 2.3 gm. per 100 cc. and globulin 3.5 gm. per 100 cc.; icteric index 36; thymol turbidity 37 units; zinc sulfate turbidity 55 units; serum bilirubin 6.42 mg. per 100 cc.

During this final admission the patient was given amethopterin in a dosage of 5 mg. daily for a period of six days. After the sixth day of amethopterin therapy, the white blood count fell to a value of 550 per cubic millimeter. No clinical improvement was noted, and the patient died on Nov. 13.

¶ Farber, S.: Personal communication.

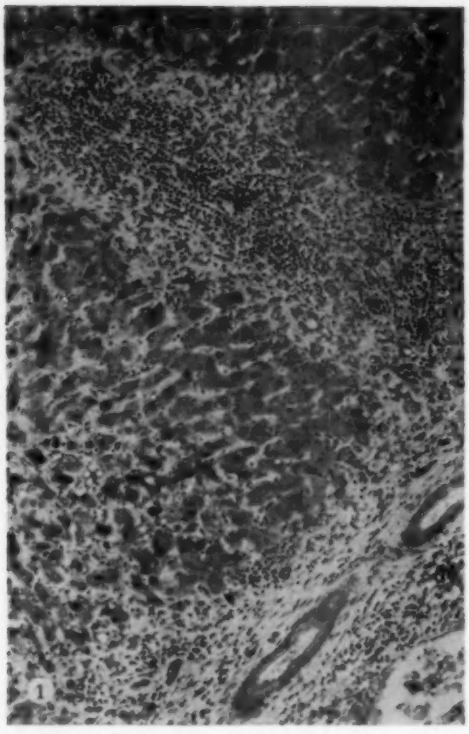


Fig. 1 (Case 1).—Liver section showing fibrosis. Romanowsky stain; reduced ⅔ from mag. × 980.

At autopsy the liver weighed 1,000 gm. and its surface was smooth and firm. On section, the parenchyma was dark brown along the branches of the portal vein, with an exaggeration of the fat on the walls of these veins at the periphery of the liver. There were areas of a grayish yellow tissue infiltrating this fat.

Microscopic examination demonstrated marked portal fibrosis, characterized by a leukemic infiltrate largely confined to the bands of connective tissue. Small proliferating bile ducts were also found in the portal areas. Centrilobular areas were not greatly altered, although there was a slight fibrosis. Brown pigment was prominent in parenchymal cells near the central vein. In occasional central zones of the lobules, fatty metamorphosis could be observed. A Van Gieson-stained section demonstrated the extent of the fibrosis in the portal areas and, to a less degree, in the center of the lobules. A few fine fibrils were seen penetrating the lobules (Figs. 1 and 2).

Case 2.—A 4-year-old white boy was admitted to the hospital in February, 1950, because of asthenia, fever, and ecchymoses. Generalized lymphadenopathy was present; the liver was palpable 8 cm. below the costal margin, and the spleen tip was palpable on deep inspiration.

Hematological examination and sternal marrow smears confirmed the diagnosis of acute leukemia.

The spleen, liver, and lymph nodes regressed completely after treatment with aminopterin, and the patient appeared to be in clinical and hematological remission.

Intermittent therapy with aminopterin was continued from April to June, 1950. In the early part of August relapse of the patient's disease appeared, and on Nov. 10 he was readmitted for further treatment.

At this time there was generalized lymphadenopathy, and the liver was found to be greatly enlarged, filling almost three-fourths of the abdomen. The spleen extended 6 cm. below the the left costal margin.

Blood Chemical Examination: Thymol turbidity 2.4 units; zinc sulfate turbidity 5.9 units; urea nitrogen 11 mg. per 100 cc.; uric acid 2.1 mg. per

Fig. 2 (Case 1).—Liver section showing fibrosis and leukemic infiltration. Van Gieson stain; reduced ⅔ from mag. × 980.

100 cc.; total protein 6.1 gm. per 100 cc., with albumin 3.1 gm. per 100 cc. and globulin 2.7 gm. per 100 cc. Sternal marrow examination revealed almost complete replacement of the marrow by blasts and immature lymphocytes.

Amethopterin, in a dose of 5 mg. intramuscularly, was administered daily for nine days beginning on Nov. 13. Numerous blood transfusions of 250 cc. each were also given. Although there was a fall in the white blood count during the first few days, no evidence of clinical improvement appeared. Therefore, alpha-peltatin, a tumor-necrotizing derivative of podophyllum resin# was administered intravenously in a dose of 2 mg., on Nov. 22, and 3 mg., on Nov. 23, while the anti-folic acid therapy was continued. On Nov. 24, 1950, the liver and spleen were found to be markedly decreased in size and the white blood cell count was 900 per cubic millimeter.

A sternal marrow examination on Dec. 29 showed an apparently normal marrow. Blood chemistry examination: total protein 6.7 gm. per 100 cc., with albumin 4.1 gm. per 100 cc. and globulin 2.6 gm. per 100 cc.; thymol turbidity 1.9 units; zinc sulfate turbidity 3 units. Serum mucoprotein 101 mg. per 100 cc.[14] Urea nitrogen 9 mg. per 100 cc.

The patient was discharged on a maintenance dose of 5 mg. of amethopterin daily.

On March 8, 1951, examination of the peripheral blood showed normal values. One month later the patient was readmitted because of increasing abdominal swelling. On physical examination the liver was large and firm and extended across the abdomen and down to the iliac crest. The spleen was palpable 6 cm. below the costal margin, and generalized lymphadenopathy was present.

Blood Chemical Examination: Urea nitrogen 18 mg. per 100 cc.; total cholesterol 219 mg. per 100 cc.; total protein 6 gm. per 100 cc., with albumin 3.5 gm. per 100 cc. and globulin 2.5 gm. per 100 cc.; alkaline phosphatase 14 Bodansky units; serum bilirubin 8.4 mg. per 100 cc.; thymol turbidity 1.8 units; zinc sulfate 5.8 units; prothrombin time 30%; serum mucoprotein 25.2 mg. per 100 cc.

Therapy with amethopterin was continued, but no change in the spleen or liver size occurred. Dilated collateral venous circulatory channels appeared over the abdomen, and then ascites and edema of both lower extremities appeared. Rectal hemorrhoids were present. The ascites and collateral abdominal venous circulation increased during the next two weeks.

On April 19 the patient developed dyspnea and cyanosis and died.

On postmortem examination the gross findings revealed prominent superficial veins over the thoracic and abdominal areas and small hemorrhoids. The liver was markedly enlarged and weighed 1,250 gm. The surface was hard but smooth.

Microscopic examination of the liver revealed the architecture to be markedly distorted by numerous fibrous strands that accentuated the lobules and gave the tissue a nodular appearance. An increase in connective tissue was found in the portal areas and communicated in such a way that all portal areas were connected, leaving the lobules of parenchymal tissue completely separate. By means of a Van Gieson stain preparation, the portal bands of connective tissue were seen to be composed of many fibrous strands, with finer fibrils penetrating into parts of the lobules. Enmeshed in these fibrous portal strands were proliferating bile capillaries, occasional lymphocytes, distended blood capillaries, and macrophages filled with brown pigment. Leukemic infiltrations were not noted anywhere. In the lobules many parenchymal cells contained a finely granular dark pigment, particularly in the peripheral areas. The sinusoids were not distended, although many contained red blood cells. Kupffer cells filled with dark pigment were prominent. Central zones of the lobules were slightly fibrotic and hyperemic but showed no marked changes. The findings in this case were considered highly typical of early nodular cirrhosis (Figs. 3 and 4).

Case 3.—A 3-year-old white boy had skeletal pains and fever for several months. On Aug. 6, 1951, a diagnosis of acute leukemia was made. The patient was admitted to the U. S. Public Health Service Hospital on Aug. 31.

Examination revealed ecchymoses and generalized lymphadenopathy. The spleen was enlarged 10 cm. below the costal margin, and the liver was 1 cm. below the costal margin.

Blood Chemical Values: Urea nitrogen 16 mg. per 100 cc.; total protein 6.8 gm., with albumin 4.2 gm. and globulin 2.6 gm. Alkaline phosphatase 10 Bodansky units; thymol turbidity 2.4 units; zinc sulfate turbidity 3 units.

Sternal marrow smear showed many blasts and lymphocytes, and the diagnosis of acute leukemia was confirmed.

Amethopterin therapy in a dose range of 5 to 7.5 mg. daily was initiated. Because of leucopenia,

References 12 and 13.

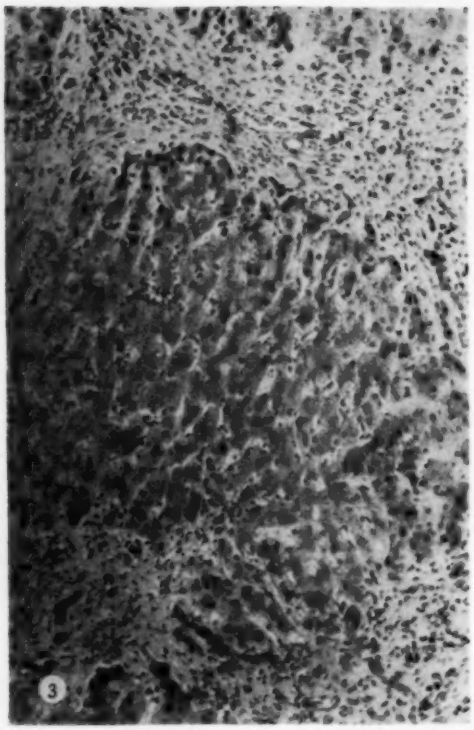


Fig. 3 (Case 2).—Liver section showing fibrotic changes. Romanowsky stain; reduced ⅔ from mag. × 980.

therapy with amethopterin was discontinued for four days and was then reinstituted. The spleen decreased in size, and the patient was clinically improved.

On Oct. 25 the spleen was no longer palpable and the liver was palpable 1 cm. below the costal margin. The patient was discharged from the hospital on a maintenance dose of amethopterin of 7.5 mg. daily.

On Dec. 20 the liver was palpable 4 cm. below the costal margin, and the spleen was 2 cm. below the costal margin. Because of the development of oral ulcerations, amethopterin was discontinued for three days and then resumed in the same dosage as before.

The patient was readmitted to the hospital because of fever and malaise on March 15, 1952. At this admission the liver and spleen were not palpable.

Blood Chemical Examination: Bilirubin 2.4 mg. per 100 cc.; thymol turbidity 14 units; zinc sulfate turbidity 18.3 units; albumin 2.5 gm. per 100 cc. and globulin 3.2 gm.

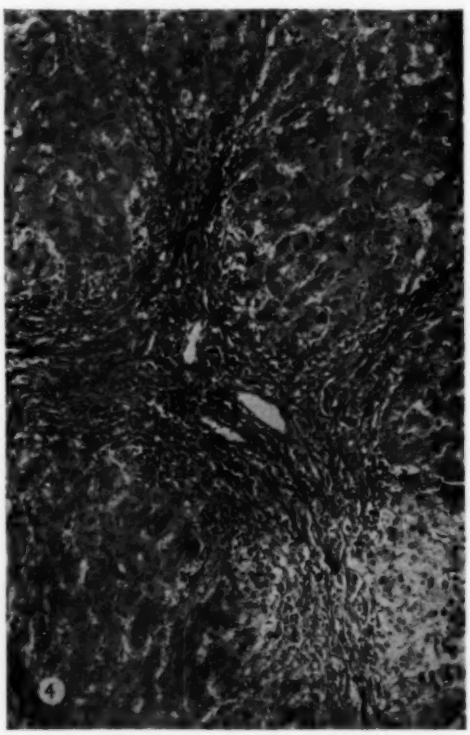
Amethopterin dosage was increased to 7.5 mg. daily. The patient did not improve, and on April 9 nasal hemorrhage began, necessitating numerous transfusions. One week later the liver was noted to be enlarging, and collateral venous channels appeared over the anterior abdominal wall, and ascites became apparent.

On May 9 paracentesis was performed and 2,500 cc. of straw-colored fluid was removed. Therapy with phenylbutazone (Butazolidin), in a dosage of 800 mg. daily, was instituted on May 14. The temperature fell to normal levels, and the patient appeared improved. On June 1 respiration ceased suddenly.

At postmortem examination 4,000 cc. of clear straw-colored fluid was present in the abdominal cavity. The liver weighed 840 gm. and had a rubbery consistency when cut.

On microscopic examination the liver had a markedly distorted architecture. Lobules were poorly defined; central vein areas were difficult to recognize because of the marked degree of change in that portion of all the lobules. Round nodules or subdivisions of lobules containing hyperplastic or regenerating liver tissue further distorted the lobular

Fig. 4 (Case 2).—Liver section demonstrating fibrotic changes. Van Gieson stain; reduced ⅔ from mag. × 980.



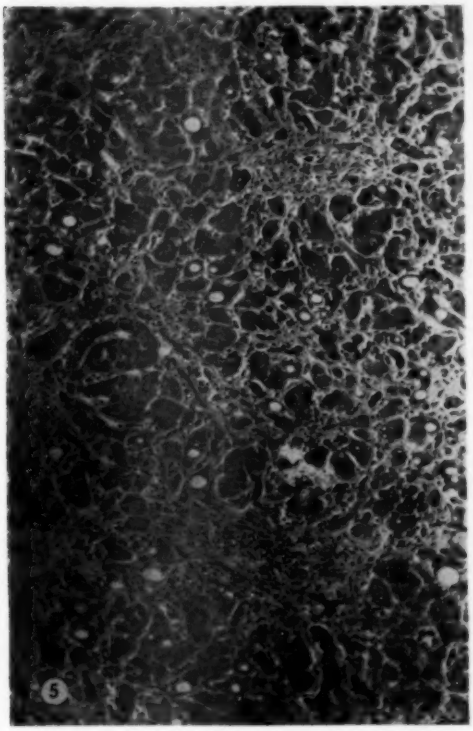


Fig. 5 (Case 3).—Liver section demonstrating fibrotic changes. Romanowsky stain; reduced ⅔ from mag. × 980.

pattern. In the centrilobular areas there was some degeneration and necrosis of liver cells. Fatty metamorphosis in some of these areas was a prominent and distorting feature. There was some bile stasis, with brown granular pigment in many parenchymal cells, but no plugged capillaries were noted. Proliferating bile capillaries were plentiful and were scattered throughout the lobules, as well as in the portal areas. The fibrous strands, as seen in the Van Gieson stain, had no consistent relation to the portal areas. Many connective tissue bands penetrated through lobules, occasionally connecting with fibrotic and necrotic central vein areas. These fibrous bands were light and rather small. Occasional fibrils were seen penetrating the lobule between cords of parenchymal cells (Figs. 5 and 6).

Case 4.—A 3½-year-old boy was well until Jan. 1, 1951, at which time he complained of sore throat and fatigue. Generalized lymphadenopathy, together with an enlarged liver and spleen, was noted. A diagnosis of acute lymphatic leukemia was made after examination of the sternal marrow.

Amethopterin therapy was started on Jan. 18, in a dose of 5 mg. daily.

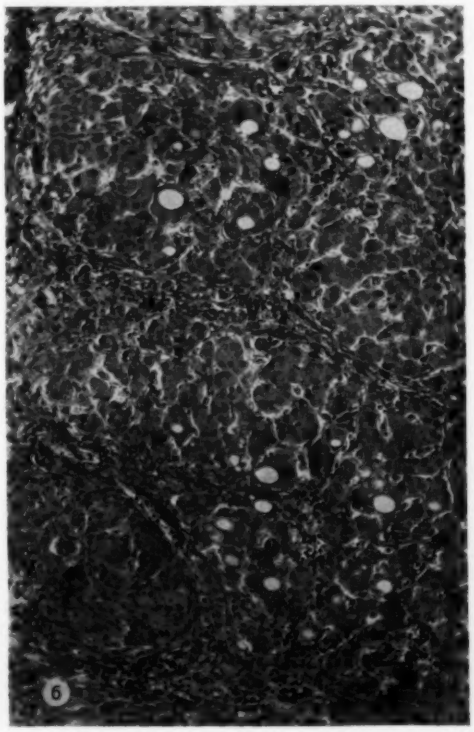
On Feb. 15 the liver edge was no longer palpable but the spleen tip was just palpable at the costal margin. The patient was in clinical and partial hematological remission. He was discharged from the hospital on maintenance amethopterin therapy, in a dosage of 5 mg. daily.

In the latter part of June, relapse appeared. The liver was enlarged 8 cm. below the costal margin, and the spleen was 8 cm. below the costal margin.

Blood Chemical Examination: Urea nitrogen 9 mg. per 100 cc.; total protein 6.5 gm., with albumin 3.9 gm. per 100 cc. and globulin 2.6 gm. per 100 cc.; phosphorus 4 mg. per 100 cc.; alkaline phosphatase 2.86 Bodansky units; bilirubin 2.5 mg. per 100 cc.; thymol turbidity 13 units; cholesterol 168 mg. per 100 cc.; zinc sulfate turbidity 12 units. Pancytopenia was present.

The abdomen became markedly distended with fluid, and edema of both lower extremities also ap-

Fig. 6 (Case 3).—Liver section showing fibrotic changes. Van Gieson stain; reduced ⅔ from mag. × 980.



peared. Erythema of the thenar and hypothenar areas of the hands was noted.

On July 23, blood chemistry values: thymol turbidity 12.4 units; zinc sulfate turbidity 21 units; albumin 2.4 gm. and globulin 3.6 gm. per 100 cc., urobilinogen 3+ in urine; blood urea nitrogen 10 mg. per 100 cc.

The patient's condition became worse, and he died in August. Consent for autopsy was not obtained.

CASE 5.—A 7-year-old girl was admitted to the U. S. Public Health Service Hospital on Oct. 16, 1950, for treatment of acute leukemia.

On physical examination the liver and the spleen were 6 cm. below the costal margin. Ecchymoses, petechiae, and lymphadenopathy were present.

On Oct. 18 treatment with amethopterin, in a dosage of 5 mg. daily, was started. The spleen and liver receded in size, so that by Oct. 30 neither was palpable. Examination of the peripheral blood revealed a return toward normal values.

Sternal marrow aspiration on Nov. 13 was reported as follows: "Marrow smear, obtained after amethopterin therapy, does not show the pattern of acute leukemia and appears apparently normal."

The patient was discharged from the hospital on Nov. 15, on a maintenance dosage of amethopterin.

In May, 1951, the spleen was markedly enlarged and the liver was palpable 4 cm. below the costal margin.

Blood Chemistry Values: Urea nitrogen 11 mg. per 100 cc.; total protein 6.4 gm. per 100 cc., with albumin 3.9 gm. and globulin 2.5 gm.; thymol turbidity 5.1 units; zinc sulfate turbidity 7.5 units; bilirubin 1.26 mg. per 100 cc.

The liver became progressively larger, and ascites and collateral abdominal venous channels appeared.

On June 9, blood chemistry values: zinc sulfate turbidity 14.1 units; thymol turbidity 11.7 units; prothrombin time 32%; serum bilirubin 1.74 mg. per 100 cc.; urea nitrogen 14 mg., total protein 6.9 gm., with albumin 3.6 gm. and globulin 3.3 gm., and mucoprotein 8 mg.[14]

Examination on June 12 revealed the spleen to be 4 cm. below the costal margin and the liver 8 cm. below the costal margin. Except for the apparent development of hepatic fibrosis, the patient appeared to be in a clinical and hematological remission. However, she died in August after a severe hemorrhage. Permission for autopsy was not obtained.

COMMENT

No single factor appears to explain satisfactorily the etiology of the hepatic fibrosis occurring in these children receiving prolonged anti-folic-acid therapy for acute leukemia. It is possible that these fibrotic changes may be due to a tissue reaction to removal of the abnormal leukemic cells as a result of therapy. Dissolution of these leukemic infiltrates which characteristically occur in the portal areas could leave areas of injury, which are later replaced by fibrous tissue.

Since all the patients in our series received numerous blood transfusions, the possibility of such hepatic changes occurring as the result of earlier homologous serum hepatitis must also be considered. None of these patients developed clinical or laboratory signs of hepatitis during the course of their illness, and so this was not considered as a likely cause of the hepatic fibrosis.

A third possibility considered is that the folic acid antagonists, by virtue of their interference with normal metabolic systems, may induce changes in the liver which stimulate the development of hepatic fibrosis. The folic acid antagonists have been reported to interfere with choline, betaine, and methionine metabolism.* Experiments conducted in animals given nutritionally deficient diets indicated that changes may be induced in the liver which will eventually lead to the production of hepatic fibrosis.† The histological changes noted were the accumulation of fat globules in the liver cells and, later, the diffuse spread of the lipid material through the liver parenchyma. As the changes progressed, strands of fibrous tissue became evident throughout the liver parenchyma. Addition of choline to the diet caused reversion of the fatty changes in the liver, but the fibrosis remained.‡ It is conceivable that interference with utilization of normal metabolites may cause changes in the human liver similar to those seen in experimental animals on low-protein low-choline diets. In a series of 50 adult patients with solid tumors, many of whom were treated with prolonged administration of aminopterin or amethopterin, changes of hepatic

* References 15 and 16.

† References 17, 18, and 19.

‡References 17 and 19.

fibrosis in the liver were not observed.[5] The administration of large doses of anti-folic-acid compounds over long periods of time, therefore, does not appear to be a sufficient cause per se for the development of hepatic fibrosis, at least in adults.

Since these hepatic changes have been noted by us only in children with acute leukemia, it is likely that a combination of mechanisms is required for the development of hepatic fibrosis. In all cases reported it could be presumed that the liver was rapidly being infiltrated by leukemic cells, which tend to occupy the portal spaces. With the onset of a remission of the disease process induced by the antimetabolites, there was decrease in liver size, which indicated a decrease in number of the invading neoplastic cells in the liver. Removal of these cells may leave small areas of hepatic injury. Continuous administration of anti-folic-acid drugs and interference with normal metabolic processes might further interfere with normal repair processes following this partial collapse of the liver stroma, and a greater and more diffuse fibrosis might be induced.

In addition, the possible factor of the greater susceptibility of the liver in young patients to metabolic antagonists of essential nutrients may have also played a part in the production of these fibrotic changes.

SUMMARY

Five of seven children ill with leukemia developed significant remissions of the disease process after treatment with aminopterin or amethopterin.

In these five children laboratory and clinical signs of hepatic fibrosis appeared after prolonged therapy with anti-folic-acid compounds. On postmorten examination of these patients there was histological evidence of extensive hepatic fibrosis.

The etiological factors causing these changes are not known. It is suggested that a combination of factors—hepatic injury following the dissolution of leukemic infiltration in the liver by treatment, failure of normal healing to take place because of interference with utilization of normal metabolites, and a greater susceptibility of the liver of young patients to metabolic antagonists—may have resulted in the development of the hepatic fibrosis.

REFERENCES

1. Farber, S. A.; Diamond, L. K.; Mercer, R. D.; Sylvester, R. F., and Wolff, J. A.: Temporary Remissions in Acute Leukemia in Children Produced by Folic Acid Antagonist, 4-Aminopteroyl-Glutamic Acid (Aminopterin), New England J. Med. **238**:787-793 (June 3) 1948.

2. Burchenal, J. H.; Karnofsky, D. A.; Kingsley-Pillers, E. M.; Southam, C. M.; Myers, W. P. L.; Escher, G. C.; Craver, L. F.; Dargeon, H. W., and Rhoads, C. P.: Effects of the Folic Acid Antagonists and 2,6-Diamino-Purine on Neoplastic Disease with Special Reference to Acute Leukemia, Cancer **4**:549-569 (May) 1951.

3. Sacks, M. S.; Bradford, G. T., and Schoenbach, E. B.: Response of Acute Leukemia to the Administration of the Folic Acid Antagonists, Aminopterin and A-Methopterin: Report of 14 Cases, Ann. Int. Med. **32**:80-115 (Jan.) 1950.

4. Smith, C. H., and Bell, W. R.: Aminopterin in Treatment of Leukemia in Children: Serial Aspirations of Bone Marrow as a Guide to Management and Appraisal of Treatment, Am. J. Dis. Child. **79**:1031-1048 (June) 1950.

5. Schoenbach, E. B.; Colsky, J., and Greenspan, E. M.: Observations on the Effects of the Folic Acid Antagonists, Aminopterin and Amethopterin, in Patients with Advanced Neoplasms, Cancer **5**:1201-1220 (Nov.) 1952.

6. Dacie, J. V.; Dresner, E.; Mollin, D. L., and White, J. C.: Acute Leukaemia Treated with A-Methopterin, Brit. M. J. **1**:784-792 (April 14) 1951.

7. Proceedings of the Second Conference on Folic Acid Antagonists in the Treatment of Leukemia, Blood (Supp.) **7**:97-190 (Jan.) 1952.

8. Taylor, S. G., III; Slaughter, D.; Preston, F. W.; Crumrine, J., and Hass, G.: Effect of Antifolic Acid Derivatives on Patients with Far-Advanced Carcinomatosis, J. Lab. & Clin. Med. **33**:1645-1646 (Dec.) 1948.

9. Thiersch, J. B.: Bone-Marrow Changes in Man After Treatment with Aminopterin, Amethopterin, and Aminoanfol, with Special Reference to Megaloblastosis and Tumor Remission, Cancer **2**:877-883 (Sept.) 1949.

10. Schoenbach, E. B.; Colsky, J., and Lewis, R. A.: Steroid Excretion in Patients Receiving Antifolic Acid Compounds, Cancer **3**:844-848 (Sept.) 1950.

11. Hanlon, D. G.; Mason, H. L., and Stickney, J. M.: Urinary Excretion of 17-Ketosteroids and Corticosteroids in Leucemia: Effect of Aminopterin (4-Amino-Pteroylglutamic Acid) on the Excretion of Corticosteroids in Acute Leucemia, J. Lab. & Clin. Med. **36**:877-886 (Dec.) 1950.

12. Greenspan, E. M.; Leiter, J., and Shear, M. J.: Effect of Alpha-Peltatin, Beta-Peltatin, and Podophyllotoxin on Lymphomas and Other Transplanted Tumors, J. Nat. Cancer Inst. **10**:1295-1333 (June) 1950.

13. Greenspan, E. M.; Colsky, J.; Schoenbach, E. B., and Shear, M. J.: Response of Patients with Advanced Neoplasm to the Intravenous Administration of Alpha-Peltatin, J. Nat. Cancer Inst. **14**: 1257-1275 (June) 1954.

14. Greenspan, E. M.; Lehman, I.; Graff, M. M., and Schoenbach, E. B.: Comparative Study of the Serum Glycoproteins in Patients with Parenchymatous Hepatic Disease or Metastatic Neoplasia, Cancer **4**:972-983 (Sept.) 1951.

15. Williams, J. N., Jr.: Effect of Aminopterin on Choline, Betaine Aldehyde, and Betaine Metabolism, Proc. Soc. Exper. Biol. & Med. **78**:206-208 (Oct.) 1951.

16. Dinning, J. S.; Keith, C. K., and Day, P. L.: Influence of Folic Acid on Methionine Metabolism, J. Biol. Chem. **189**:515-520 (April) 1951.

17. Lillie, R. D.; Ashburn, L. W.; Serbrell, W. H.; Daft, F. S., and Lowry, J. V.: Histogenesis and Repair of the Hepatic Cirrhosis in Rats Produced on Low Protein Diets and Preventable with Choline, Pub. Health Rep. **57**:502-508 (April 3) 1942.

18. Rich, A., and Hamilton, J. D.: Experimental Production of Cirrhosis of the Liver by Means of a Deficient Diet, Johns Hopkins Hosp. **66**:185-198 (March) 1940.

19. Hartroft, W. S., and Ridout, J. H.: Pathogenesis of the Cirrhosis Produced by Choline Deficiency: Escape of Lipid from Fatty Hepatic Cysts into the Biliary and Vascular Systems, Am. J. Path. **27**:951-989 (Nov.-Dec.) 1951.

MORPHOLOGICAL STUDIES OF HYALINE MEMBRANES IN THE NEWBORN INFANT

W. S. GILMER, M.D.
and
ALBERT M. HAND, M.D., Memphis

HYALINE membranes in the newborn infant were recognized by Hoccheim [1] in 1903 and were first described in the United States by Johnson [2] in 1923. Sporadic studies have appeared since in the literature, but only in recent years has hyaline membrane with atelectasis attained the prominence it deserves as a cause of neonatal death. For example, Potter [3] stated: "It was present as the only pathological change in 40% of all infants weighing from 1000 to 2500 grams who died between 1939 and 1949 at the Chicago-Lying-In Hospital." Miller and Jennison [4] reported that hyaline membrane disease was the cause of death in 90% of the infants weighing over 1000 gm. who were born alive yet died during the first 48 hours of life if one eliminates those who had clinically recognized defects or disease and those whose mothers had a serious complication of pregnancy. In a review of the literature, Tran-Dinh-De and Anderson [5] found that in 2469 autopsies of liveborn infants weighing over 1000 gm., 21.3% had pulmonary hyaline-like membranes. It has been estimated that 25,000 infants die yearly in the United States as a result of this entity.

Clinically, the condition characteristically occurs in premature infants, in those delivered by Caesarean section,[6] and in infants of diabetic mothers. In many instances, after a brief period of apparent well-being the infant develops sternal retraction, respiratory distress, and cyanosis, which may be intermittent at first but later is constant and becomes progressively severer. We have examined the clinical records and courses of 47 newborn infants who had an autopsy diagnosis of hyaline membranes during 1951, 1952, and 1953 at the John Gaston Hospital, and, in our experience, the period of "apparent normalcy" during the first hours of life of these infants is not well defined, as Miller and Jennison [4] have previously noted. Death occurred between 2 and 36 hours in our cases. If the infant survives 48 hours after birth, recovery is the rule, unless the situation is complicated by pneumonia, which often leads to death [3] in this condition.

The histological description of the lungs by Johnson and Meyer [7] (in 1925) has not been altered appreciably in subsequent reports. The terminal portion of the respiratory segments of the lungs, the alveolar ducts, and the atria are "lined" by a granular eosinophilic hyaline-like material, and the intervening parenchyma is atelectatic and markedly congested (Fig. 1). The congestion has been emphasized by Potter,[3] Farber and Wilson,* and others, as responsible for the red-purple appearance characteristic of the lungs at autopsy.

The numerous studies on the etiology and pathogenesis of pulmonary hyaline membranes have been analyzed by Tran-Dinh-De and Anderson [5] in their comprehensive review of the subject. Histochemical studies, to be discussed below, failed to identify the specific nature of the material or to indicate its source. † The significance of intrauterine respiratory-like movements of the fetus in the pathogenesis of this condition has been widely discussed, but no relationship has

Aided in part by U. S. P. H. S. Grant No. E-620.

Playtex Park Research Institute Fellow (Dr. Hand).

From the Division of Pathology and Bacteriology, the University of Tennessee, and the City of Memphis Hospitals.

* References 8 and 9.

† References 8 through 12.

been established.[13] Experimental reproduction of the condition has been attempted, utilizing many methods, the most successful of which being cervical vagotomy ‡ and oxygen poisoning.[16] Though hyaline-like membranes may be produced by the above methods, these membranes are not associated with the widespread atelectasis characteristic of the condition in the human.[5]

The source of the hyaline material has been the subject of lively debate. The suggestion that it is derived from aspirated epidermal cells and fat appears to have been disproved. § Rosenthal,[19] in 1935, gave the condition the name "desquamative anaerosis." Tregillus,[20] in 1951, revived this concept, using the term "asphyxial membrane,"

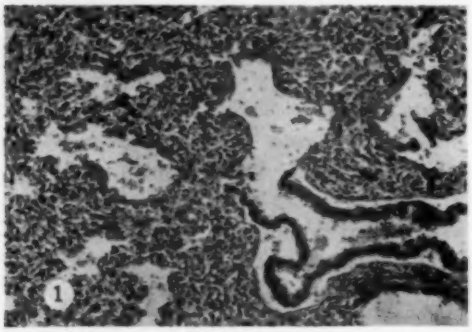


Fig. 1.—Hyaline membranes in the newborn infant. The hyaline material is closely applied to the walls of the alveolar ducts and atria. The alveoli are collapsed. Hematoxylin and eosin; reduced ⅓ from × 300.

and attributed it to necrosis and hyalinization of bronchiolar epithelium. It is clear that those who have investigated the condition may be separated into two diametrically opposed groups as follows: (1) those who feel, as did Ahvenainen [11] and Blystad and co-workers,[12] that it results from aspiration of amniotic fluids and (2) others, such as Miller and Jennison,[4] who maintain that it is derived from the circulating blood. Consequently, the problem at present focuses on the demonstration of the source of the hyaline material seen in the lungs of newborn infants.

‡ References 14 and 15.

§ References 17 and 18.

Largely ignored have been anatomical studies upon the respiratory segment of the lung. The recent investigation of Low ‖ demonstrating by electron microscopy the presence of a basement membrane, characterized by him as "epithelial," in the terminal portions of the respiratory segment of the lungs in animals and humans, may be expected to shed new light upon this complicated problem. Low's concept, not previously accepted by leading investigators,¶ confirms the work of a number of workers, such as Miller [26] and Sprunt and others.#

The present investigation concerns histological and histochemical studies made upon the respiratory segment of lungs of newborn infants dying with pulmonary hyaline membranes and on those of infants and children dying with other conditions, as outlined below.

MATERIAL AND METHODS

The following autopsy cases were selected for study: (1) 10 newborn infants with hyaline membranes; (2) an 8-year-old girl with rheumatic fever; (3) a 3-month-old boy with bronchopneumonia; (4) a 2180 gm. premature infant who died 20 minutes after breech delivery; (5) a 1010 gm. premature infant who died suddenly at the age of one day, and (6) a 1250 gm. stillborn male infant, with premature separation of the placenta.

The lungs were fixed in 10% formalin and processed in the usual manner, with the exception of those sections used for acetylation studies, which were fixed in chilled acetone. The following staining techniques were used: (1) hematoxylin and eosin, (2) periodic acid-Schiff reagent reaction (P. A. S.),[27] (3) the Ritter and Oleson technique (R. and O.), (4) P. A. S. after malt diastase digestion, (5) Wilder's silver stain, (6) phosphotungstic acid-hematoxylin stain, (7) the MacCallum stain, (8) toluidine blue stain, (9) the Feulgen reaction, (10) the Millon reaction, (11) the acetylation technique of McManus,[28] and (12) Sudan IV stain.

Examination of the tissues was done with an ordinary light microscope, using at times a magnification at about the limit of its resolving power (× 1,649).

‖ References 21 and 22.

¶ References 23 through 25.

Sprunt and others, in Pulmonic Alveolar Epithelium.[24]

RESULTS

1. *Histochemical Studies on Hyaline Membranes.*—Our results confirm the previous reports that fat is present in some, but not all, hyaline membranes in the newborn infant and would seem to be of no significance.* The MacCallum stain demonstrates that it is not fibrin. Although occasional small fragments of material within the membrane are found in the Feulgen reaction, deoxyribose nucleic acid is not a consistent component of the material. The membranes are not metachromatic, indicating that, if hyaluronic acid is present, it is in a depolymerized form. That protein is a major constituent is indicated by the Millon reaction. The material is strongly positive with the periodic acid-Schiff reagent stain (P. A. S.), generally accepted as indicating the presence of polysaccharides, but this stain is in no sense a specific histochemical test. Accordingly, we felt that more accurate identification of the polysaccharides was indicated. Sections were incubated with malt diastase, which was found to have no effect upon the P. A. S. staining of the hyaline membranes while abolishing the staining of bronchiolar cells and the squamous cells in the aspirated vernix. Not only does this demonstrate that glycogen is not a constituent of the hyaline membranes in the newborn infant, but it provides another method to distinguish hyaline membranes from aspirated vernix, which, to our knowledge, has not been previously described.

The acetylation technique of McManus [25] has been used to distinguish carbohydrates containing 1,2-glycol linkages from other P. A. S.-positive substances by blocking the hydroxyl groups and preventing the formation of aldehydes which are stained by the Schiff reagent. When this technique is applied to hyaline membranes in the newborn infant, they are no longer stained by the P. A. S. method, indicating that the carbohydrate present is one with a 1,2-glycol linkage.

2. *Lungs of Infants and Older Children Dying with Conditions Other Than Hyaline Membranes.*—It is well recognized, as shown by Miller [26] and Sprunt,† that in certain pathological conditions there becomes apparent in the respiratory segment of the lung a lining or basement membrane, demonstrated in our case of rheumatic fever (Fig. 2). With the highest magnification obtainable with our light microscope, we were able to demonstrate a similar, although less conspicuous, basement membrane in each of the six types of cases studied (which are listed above). This basement membrane can be seen distinctly as separate from the capillary walls in the alveolar ducts and atria,

* References 4 and 16 through 18.

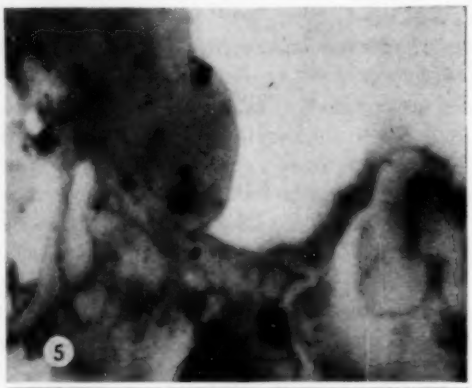
Fig. 2.—Rheumatic fever. The epithelial lining is shown clearly, especially where it is detached from the capillaries of the septae due to shrinkage during fixation. Wilder's silver stain; reduced ⅓ from × 1300.

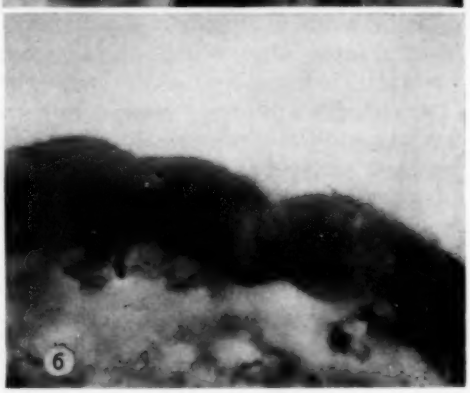
Fig. 3.—Premature infant, weighing 2,180 gm., died 20 minutes after breech extraction. The epithelial lining may be seen coursing parallel to the basement membrane of a capillary loop and continuing beyond the end of the loop. An epithelial cell is seen in continuity with the epithelial lining membrane. Wilder's silver stain; reduced ⅓ from × 1300.

† Sprunt, in Pulmonic Alveolar Epithelium.[24]

extending in a continuous fashion throughout the respiratory segment overlying the highly vascular parenchyma (Fig. 3). Continuity with alveolar lining cells also may be demonstrated, although these in no sense constitute a continuous cellular layer over the basement membrane.

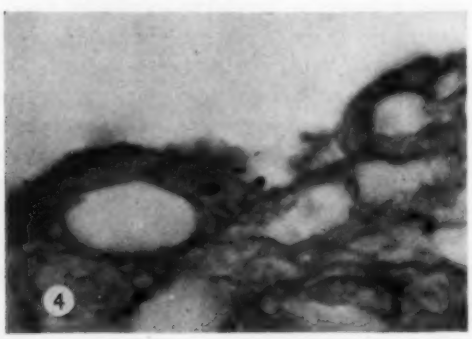
3. *Lungs of Newborn Infants Dying with Hyaline Membranes.*—In these lungs a basement membrane was again demonstrated. With the Ritter and Oleson stain this lining is dark blue, in contrast to the reddish-pink hyaline material. It became apparent early in this study that, in many instances, the hyaline material was present within, or beneath, this lining. One can trace the continuous blue-staining element, which we have characterized as the alveolar basement membrane, in apposition with the endothelial basement membrane of the capillary walls, also staining blue and appearing as loops with arcs of varying sizes. A minimal amount of pink-staining material then appears between the two membranes, both in a segmental distribution and diffusely (Fig. 4). This increases in amount, elevates at least portions of the outer membrane, and separates it from the capillary walls, so that the endothelial and alveolar basement membranes may be separated by as much as 10 to 50 μ or more, (Figs. 5 and 6). However, it is apparent that, as the accumulation of the material becomes excessive, the outer element is fragmented, so that a continuous layer over the massive hyaline accumulations is not always present. It is in such cases that squamous cells may be found within the hyaline material, but in no instance in the cases studied was such an admixture observed when the basement membrane over the hyaline material was intact. The time sequence in the formation and size of the membrane is not directly correlated with the duration of the disease, insofar as we can determine.





Figs. 5 and 6.—Hyaline membranes in the newborn infant. A more massive accumulation of material is seen here, with both overlying epithelial membrane and underlying capillaries well demonstrated. Ritter and Oleson stain; reduced ⅓ from × 1300.

Fig. 4.—Hyaline membranes in the newborn infant. This represents an early state in the development where small, more or less focal accumulation of the material may be seen lying over the capillary loops and, in turn, being overlain by at least part of the epithelial membrane. Ritter and Oleson stain; reduced ⅓ from × 1300.



COMMENT

Histochemical studies, although not completed, have failed thus far to indicate the exact nature, or the source, of the hyaline substance present in hyaline membranes of

the newborn infant. It is accepted that the primary constituents are proteins and carbohydrates. We have been able to show that the carbohydrate (or polysaccharide) moiety contains a 1,2-glycol linkage. Malt diastase digestion not only demonstrated the absence of glycogen in the hyaline material but proved to be a method of distinguishing it from aspirated vernix.

The presence or absence of an epithelial lining in the respiratory segment of normal human lungs has been a disputed subject since its presence was first asserted by von Kölliker.[29] In few fields of histology are the proponents of opposing views so adamant in their stands. Among those who contend that such a structure exists have been Miller,[26] Bremer,‡ Sprunt,§ Bensley and Bensley,[30] and others. Prominent among the opponents have been Loosli, Adams, and Thornton[23]; Palmer‖; Fried,¶ and, recently, Potter and Loosli.[25] Potter[3] epitomized the view of the latter school of thought in the statement, "The walls of the alveoli are composed of capillaries, a fine network of elastic fibers and very few connective tissue cells." While it may be admitted that this situation could be assumed because of the specialized function of the capillaries of the lung, such a situation would be unique, for nowhere else in the body are capillaries found in direct contact with open space which is either air- or fluid-containing.

Recently, Low,[21] utilizing electron microscopy, demonstrated the presence of such a structure as we have described, to which he referred as "pulmonary epithelium." In his opinion, the cytoplasm of the epithelial cells becomes attenuated to form a complete lining of the alveolar wall, just as attenuation of the cytoplasm of the endothelial cells forms the wall of the capillaries, e. g., the capillary basement membrane. In a more recent communication, Low[22] stated that the same situation was found in the lungs of rabbit, guinea pig, dog, and human.

‡ Bremer, in Pulmonic Alveolar Epithelium.[24]
§ Sprunt, in Pulmonic Alveolar Epithelium.[24]
‖ Palmer, in Pulmonic Alveolar Epithelium.[24]
¶ Fried, in Pulmonic Alveolar Epithelium.[24]

We do not propose to enter into a debate as to whether or not the alveolar lining cells are of an "epithelial" nature. That they exist is beyond doubt. The essential feature of this discussion is that there does exist a lining basement membrane in the respiratory segment of the lung and that Low's suggestion that it represents an "attenuation" of the lining cells seems plausible.

The objection may be raised that the basement membranes shown in our illustrations are more prominent than those which Low illustrates. This, of course, must apply to both the alveolar and endothelial membranes, which, in our cases, are of approximately the same magnitude. We feel that it is not possible to compare these structures as seen in animals and adults to those of premature children.

As Jones[31] pointed out in his studies on human glomerulonephritis, there exists throughout the body a fundamental capillary-connective-tissue-epithelial relationship, in which epithelium lying against connective tissue has a basement membrane and between this epithelial basement membrane and the capillary basement membrane connective substances (ground substance and/or the products thereof) are present. We feel that the existence of this same fundamental relationship in the human lung has been revealed.

Inasmuch as reported studies on hyaline membranes in lungs of newborn infants generally have accepted the opinion of those who contended that lining cells and a basement membrane did not exist in the respiratory segment of the lung, our findings may be of significance in the pathogenesis of this condition. As pointed out, attention was focused on the source of the hyaline material, specifically, whether it is exogenous or endogenous in origin.

We feel that the present study has indicated that, in the cases studied, the material is endogenous in origin, because of its location beneath the respiratory basement membrane. There are, then, the following possibilities: 1. The material is derived from the blood stream, presumably as a result of capil-

lary damage. 2. A second possibility, and one which should be considered seriously, is that the basement membrane itself, representing a continuation of the cytoplasm of the lining cells, may undergo alterations, so that the hyaline membranes actually represent a change within the basement membrane. 3. Finally, changes may occur in the connective substances between the two basement membranes to produce the accumulation of hyaline material.

SUMMARY

A basement membrane lines the respiratory segment of the human lung which may be distinguished from the capillary basement membrane. Between the two there is a connective tissue space.

In hyaline membranes in the newborn infant the offending material may be found beneath this basement membrane.

It is believed that this indicates the endogenous origin of the material, which (1) may be derived from the blood stream, (2) may represent a change within the respiratory basement membrane, or (3) may represent a change in the connective tissue substances between the two membranes.

Mr. John Dickson prepared the photographic illustrations.

REFERENCES

1. Hoccheim, K.: Herrn Geh. Medicinabrat Dr. Johannes Orth zur Feier seines 25-jährigen Professoren Jubiläums Gewidmet, Berlin, 1903,

2. Johnson, W. C.: Pneumonia in Newborn Infants with Lesions Resembling Influenza, Proc. New York Path. Soc. **23**:138, 1923.

3. Potter, E. L.: Pathology of the Fetus and Newborn, Chicago, Year Book Publishers, Inc., 1952, Chap. 15, pp. 244, 252.

4. Miller, H. C., and Jennison, M. H.: Study of Pulmonary Hyaline-like Material in 4,117 Consecutive Births: Incidence, Pathogenesis and Diagnosis, Pediatrics **5**:7, 1950.

5. De, T. D., and Anderson, G. W.: Hyaline-like Membranes Associated with Diseases of the Newborn Lungs: Review of the Literature, Obst. & Gynec. Surv. **8**:1, 1953.

6. Arey, J. B., and Dent, J.: Causes of Fetal and Neonatal Death with Special Reference to Pulmonary and Inflammatory Lesions, J. Pediat. **42**:1 and 205, 1953.

7. Johnson, W. C., and Meyer, J. R.: Pneumonia in the Stillborn and Newborn, Am. J. Obst. & Gynec. **9**:151, 1925.

8. Farber, S., and Wilson, J. L.: Hyaline Membrane in the Lungs: I. Descriptive Study, Arch. Path. **14**:437, 1932.

9. Farber, S., and Wilson, J. L.: Hyaline Membrane in the Lungs: II. Experimental Study, Arch. Path. **14**:450, 1932.

10. Farber, S., and Sweet, L. K.: Amniotic Sac Contents in Lungs of Infants, Am. J. Dis. Child. **42**:1372, 1931.

11. Ahvenainen, E. K.: On Membranes ("Vernix Membranes") Caused by Amniotic Fluid in Lungs of Newborn and on Interstitial Emphysema, as Well as Spontaneous Pneumothorax, Ann. med. int. Fenniae **36**:367, 1947.

12. Blystad, W.; Landing, B. H., and Smith, C. A.: Pulmonary Hyaline Membranes in Newborn Infants: Statistical, Morphologic, and Experimental Study of Their Nature, Occurrence and Significance, Pediatrics **8**:5, 1951.

13. Intrauterine Respiration: Report of the Fifth M & R Pediatric Research Conference, W. E. Nelson, Chairman, edited by James B. Orey, Columbus, Ohio, M & R Laboratories, 1953, pp. 17-44.

14. Farber, S.: Studies on Pulmonary Edema: Consequences of Bilateral Cervical Vagotomy in Rabbit, J. Exper. Med. **66**:397, 1937.

15. Miller, H. C.; Behrle, F. C., and Gibson, D. M.: Comparison of Pulmonary Hyaline Membranes in Vagotomized Rabbits with Those in Newborn Infants, Pediatrics **7**:611, 1951.

16. Bruns, P. D., and Shields, L. V.: Pathogenesis and Relationship of the Hyaline-Like Pulmonary Membrane to Premature Neonatal Mortality, Am. J. Obst. & Gynec. **61**:953, 1951.

17. Miller, H. C., and Hamilton, T. R.: Pathogenesis and Relationship of the Hyaline-like Pul-Aspiration Pneumonia in Stillborn and Newborn Infants, Pediatrics **3**:735, 1949.

18. Reiner, C.: Pulmonary Hyaline Membranes: Chemical Nature, in Intrauterine Respirations: Report of the Fifth M & R Pediatric Research Conference, W. E. Nelson, Chairman, edited by James B. Orey, Columbus, Ohio, M & R Laboratories, 1953, pp. 61-63.

19. Rosenthal, M.: Pulmonary Lesions Associated with Intrauterine Asphyxia, J. Pediat. **6**:71, 1935.

20. Tregillus, J.: Asphyxial Membrane in the Lungs of Liveborn Infants, J. Obst. & Gynaec. Brit. Emp. **58**:406, 1951.

21. Low, F. N.: Electron Microscopy of the Rat Lung, Anat. Rec. **113**:437, 1952.

22. Low, F. N.: Pulmonary Alveolar Epithelium of Laboratory Mammals and Man, Anat. Rec. **117**: 241, 1953.

23. Loosli, C. G.; Adams, W. E., and Thornton, T. M., Jr.: Histology of the Dog's Lung Following Experimental Collapse: With Special Reference to the Nature of the Alveolar Lining, Anat. Rec. **105**: 697, 1949.

24. Pulmonic Alveolar Epithelium: A Round Table Conference, C. C. Macklin, Chairman, J. Thoracic Surg. **6**:82, 1936.

25. Potter, E. L., and Loosli, C. G.: Prenatal Development of the Human Lung, A. M. A. Am. J. Dis. Child. **82**:226, 1951.

26. Miller, W. S.: The Lung, Ed. 2, Springfield, Ill., Charles C Thomas, Publisher, Chap. 4, pp. 39-73.

27. McManus, J. F. A.: Histological and Histochemical Uses of Periodic Acid, Stain Tech. **23**:99, 1948.

28. McManus, J. F. A., and Cason, J. E.: Carbohydrate Histochemistry Studied by Acetylation Techniques: I. Periodic Acid Methods, J. Exper. Med. **91**:651, 1950.

29. von Kölliker, A.: Zur Kenntniss des Baues der Lunge des Menschen, Verhandl. phys.-med. Gesellsch. Wurzb. **16**:1, 1881.

30. Bensley, R. D., and Bensley, S. H.: Studies of the Lining of the Pulmonary Alveolus of Normal Lungs of Adult Animals, Anat. Rec. **64**:41, 1935.

31. Jones, D. B.: Glomerulonephritis, Am. J. Path. **29**:33, 1953.

News and Comment

GENERAL NEWS

Course on Parasitic Infections.—A short intensive course on the laboratory diagnosis and pathology of parasitic infections will be presented Aug. 15-27, 1955, at the Louisiana State University School of Medicine, New Orleans.

The course is designed primarily for pathologists and technologists. However, general practitioners, internists, pediatricians, gastroenterologists, and physicians engaged in the practice of public health and tropical medicine who are interested in the laboratory diagnosis of parasitic infections are welcome to attend. The instruction and training will be of assistance to pathologists who are preparing for board examinations, to pathologists and physicians who are responsible for the diagnosis of parasitic infections in their laboratories, and to technologists engaged in this specialty.

The course will include lectures, extensive demonstrations, films, and supervised individual laboratory study. Emphasis will be placed upon the practical aspects of laboratory diagnosis of common parasitic infections, including training in stool examination and stool concentration techniques. Abundant material from patients with parasitic diseases endemic in this area will be available for examination. Comprehensive slide sets containing parasitic organisms in tissue of Microbiology, Louisiana State University School of Medicine, 1542 Tulane Ave., New Orleans 12.

Registrants should bring their microscopes, equipped with mechanical stages, and their microscope lamps. A limited number of places will be available. The fee for the course is $50.

Persons interested in attending this course may write to Dr. Clyde Swartzwelder, Department sections will be studied. Library facilities are available. The medical school building is air conditioned.

PERSONAL

Dr. Boyd Visiting Professor at Ohio State.—Dr. William S. Boyd served as Visiting Professor of Pathology at Ohio State University College of Medicine during January, 1955.

Appointment for Dr. Lewis Thomas.—Dr. Lewis Thomas, formerly professor of pediatrics and internal medicine, University of Minnesota Medical School, has been appointed Chairman of the Department of Pathology, New York University-Bellevue Medical Center. Dr. Thomas replaces Dr. W. C. Von Glahn, who has recently retired.

PLASMA SPECIFIC GRAVITY CHANGES IN SUDDEN DEATHS

Observations with Specific Reference to Drowning

HENRY C. FREIMUTH, Ph.D., Baltimore
and
HENRY E. SWANN Jr., M.S., Army Chemical Center, Md.

IN A RECENT article [1] a study of the blood changes in a number of human drowning deaths was reported. In that study comparison was made of blood from the left and right atria, and the following determinations were made: whole-blood chloride, plasma chloride, hematocrit, plasma specific gravity, hemoglobin, sodium, potassium, and total protein. These determinations were made on blood obtained from 39 human drowning deaths and from 10 additional persons whose deaths were due to causes other than drowning. It was concluded from this study that the diagnosis of death due to drowning based on the difference between whole-blood or plasma chloride concentrations in the left and right atria, as reported by Gettler,[2] is less reliable than a diagnosis based on the determination of plasma specific gravity difference in the two sides of the heart. In all of the 39 drowning deaths reported, the specific gravity of the left atrial plasma was less than that of the right atrial plasma, while the reverse condition prevailed in the 10 nondrowning deaths.

Because of the relatively few control cases, as well as drowning cases, reported in the above study, it was decided to investigate further the difference in plasma specific gravity of the left and right atria in additional drowning deaths and in a larger group of control cases involving deaths from a variety of causes.

After making determinations in several control cases, we encountered a death due to brain injury in which the specific gravity of the left heart plasma was lower than that of the right. The difference (left-right) was —0.0005. This case is listed as Case 247 in Table 2. However, it was observed that the right heart plasma was hemolyzed to a greater degree than that of the left atrium. Hemoglobin determinations on the two samples by the benzidine procedure [3] showed 510 mg. per 100 ml. in the right heart plasma and 280 mg. per 100 ml. in the left heart plasma. The specific gravity of plasma, as determined by the falling drop method [4] or the copper sulfate method,[5] may be corrected for the contribution to it of hemoglobin resulting from hemolysis by subtracting one unit from the fourth decimal place for each 10 mg. per 100 ml. of hemoglobin. This correction was made in the cited case, resulting in a difference of left heart gravity minus right heart gravity of + 0.0018.

The above observation having been made, the cases first reported [1] were reevaluated. Corrections were made in these cases for the effect of hemoglobin on the specific gravity. The original and corrected values are shown in Tables 1 and 2, in which the cases are designated by numbers from 1 to 100. The cases designated by numbers greater than 100 are the newly considered ones.

COMMENT

As originally reported, the diagnosis of drowning was based upon the algebraic difference between the specific gravity of left

From the Office of the Chief Medical Examiner, State of Maryland, and the Department of Legal Medicine, University of Maryland School of Medicine and College of Physicians and Surgeons (Dr. Freimuth), and Chemical Corps Medical Laboratories (Mr. Swann).

TABLE 1.—*Plasma Specific Gravity Differences in Human Deaths by Drowning**

Case No.	Specific Gravity			Hemoglobin, Mg./100 Ml.		Corrected Specific Gravity		
	L	R	L-R	L	R	L	R	L-R
1	1.0305	1.0312	— .0007	400	413	1.0265	1.0271	— .0006
2	1.0322	1.0341	— .0019	...	298	.....	1.0311	.....
3	1.0342	1.0364	— .0022	...	...	.....	.....	.....
4	1.0286	1.0432	— .0146	263	365	1.0261	1.0395	— .0134
5	1.0315	1.0350	— .0035	83	11	1.0307	1.0349	— .0042
6	1.0264	1.0313	— .0049	138	90	1.0250	1.0304	— .0054
7	1.0265	1.0391	— .0126	900+	183	1.0175	1.0373	— .0198
8	1.0284	1.0293	— .0019	115	106	1.0272	1.0282	— .0010
9	1.0320	1.0337	— .0017	...	...	.....	.....	.....
10	1.0316	1.0368	— .0052	...	63	.....	1.0362	.....
11	1.0362	1.0397	— .0035	...	...	.....	.....	.....
12	1.0236	1.0311	— .0075	...	...	.....	.....	.....
20	1.0308	1.0328	— .0020	22	54	1.0306	1.0323	— .0017
21	1.0229	1.0289	— .0060	...	5	.....	.....	.....
22	1.0286	1.0357	— .0071	10	10	1.0285	1.0356	— .0071
23	1.0273	1.0291	— .0018	121	54	1.0261	1.0286	— .0025
24	1.0234	1.0347	— .0113	...	...	.....	.....	.....
25	1.0210	1.0283	— .0073	30	86	1.0207	1.0274	— .0067
26	1.0301	1.0319	— .0018	...	...	.....	.....	.....
27	1.0358	1.0428	— .0070	...	...	.....	.....	.....
28	1.0222	1.0380	— .0158	...	...	.....	.....	.....
29	1.0246	1.0308	— .0062	15	19	1.0244	1.0306	— .0063
30	1.0312	1.0323	— .0011	113	125	1.0301	1.0310	— .0009
31	1.0291	1.0348	— .0057	226	148	1.0268	1.0333	— .0065
32	1.0302	1.0345	— .0043	83	15	1.0294	1.0343	— .0049
33	1.0278	1.0384	— .0106	234	148	1.0255	1.0369	— .0114
34	1.0221	1.0362	— .0141	76	210	1.0213	1.0341	— .0128
35	1.0203	1.0311	— .0108	...	...	.....	.....	.....
36	1.0317	1.0349	— .0032	...	...	.....	.....	.....
37	1.0277	1.0307	— .0030	...	...	.....	.....	.....
38	1.0246	1.0352	— .0079	...	...	.....	.....	.....
39	1.0251	1.0293	— .0042	...	...	.....	.....	.....
40	1.0285	1.0298	— .0013	...	...	.....	.....	.....
41	1.0263	1.0363	— .0100	...	...	.....	.....	.....
42	1.0372	1.0377	— .0005	...	...	.....	.....	.....
43	1.0273	1.0338	— .0065	...	...	.....	.....	.....
90	1.0319	1.0468	— .0149	...	...	.....	.....	.....
91	1.0275	1.0438	— .0163	...	...	.....	.....	.....
92	1.0289	1.0328	— .0039	...	...	.....	.....	.....
101	1.0238	1.0408	— .0170	500	100	1.0188	1.0398	— .0210
102	1.0255	1.0328	— .0073	15	10	1.0253	1.0327	— .0074
103	1.0282	1.0305	— .0023	5	0	1.0281	1.0305	— .0024
104	1.0214	1.0313	— .0099	39	158	1.0210	1.0297	— .0087
105	1.0290	1.0293	— .0003	42	39	1.0286	1.0289	— .0003
106	1.0284	1.0324	— .0040	39	35	1.0280	1.0320	— .0040
107	1.0260	1.0330	— .0070	15	19	1.0258	1.0328	— .0070
108	1.0319	1.0343	— .0024	...	...	.....	.....	.....
109	1.0298	1.0343	— .0045	...	...	.....	.....	.....
110	1.0268	1.0303	— .0035	...	...	.....	.....	.....
111	1.0301	1.0314	— .0013	...	...	.....	.....	.....
112	1.0276	1.0311	— .0035	...	...	.....	.....	.....
113	1.0230	1.0279	— .0049	...	...	.....	.....	.....
114	1.0191	1.0343	— .0152	...	...	.....	.....	.....
115	1.0207	1.0351	— .0144	...	...	.....	.....	.....
116	1.0287	1.0309	— .0022	...	...	.....	.....	.....
117	1.0298	1.0346	— .0048	...	...	.....	.....	.....
118	1.0322	1.0382	— .0060	...	...	.....	.....	.....
119	1.0282	1.0300	— .0018	10	92	1.0281	1.0291	— .0010
120	1.0276	1.0326	— .0050	...	...	.....	.....	.....
121	1.0256	1.0292	— .0036	...	...	.....	.....	.....
122	1.0238	1.0330	— .0092	...	...	.....	.....	.....
123	1.0273	1.0320	— .0047	...	...	.....	.....	.....
124	1.0290	1.0286	+ .0004	102	21	1.0280	1.0284	— .0004
125	1.0224	1.0341	— .0117	...	...	.....	.....	.....
126	1.0268	1.0295	— .0027	...	...	.....	.....	.....
127	1.0217	1.0315	— .0098	...	...	.....	.....	.....
128	1.0271	1.0306	— .0035	...	...	.....	.....	.....
129	1.0284	1.0361	— .0077	...	...	.....	.....	.....
130	1.0226	1.0261	— .0035	...	...	.....	.....	.....
131	1.0195	1.0308	— .0113	...	...	.....	.....	.....
132	1.0204	1.0212	— .0008	...	...	.....	.....	.....
133	1.0252	1.0330	— .0078	...	...	.....	.....	.....
134	1.0280	1.0312	— .0032	...	...	.....	.....	.....
135	1.0221	1.0307	— .0086	42	86	1.0217	1.0298	— .0081
136	1.0263	1.0292	— .0029	...	...	.....	.....	.....
137	1.0299	1.0314	— .0015	...	...	.....	.....	.....
138	1.0251	1.0311	— .0060	...	...	.....	.....	.....
139	1.0261	1.0309	— .0048	...	...	.....	.....	.....
140	1.0254	1.0332	— .0078	...	...	.....	.....	.....
141	1.0262	1.0283	— .0016	...	...	.....	.....	.....

* L indicates left atrium; R, right atrium.

TABLE 2.—*Plasma Specific Gravity Differences in Human Cases of Sudden Death**

Case No.	Specific Gravity			Hemoglobin, Mg./100 Ml.		Corrected Specific Gravity		
	L	R	L-R	L	R	L	R	L-R
Cardiac Failure								
45	1.0372	1.0346	+ .0026	...	13	.....	1.0345	.....
46	1.0315	1.0302	+ .0013	355	16	1.0279	1.0300	— .0021
47	1.0322	1.0308	+ .0014	48	5	1.0317	1.0308	+ .0009
201	1.0318	1.0378	— .0060	315	900	1.0286	1.0288	— .0002
202	1.0360	1.0383	— .0023	11	20	1.0359	1.0381	— .0022
203	1.0355	1.0340	+ .0015	180	...	1.0337	.....	.....
204	1.0295	1.0292	+ .0003	81	156	1.0287	1.0276	+ .0011
205	1.0308	1.0315	— .0007	1000+	500	1.0208	1.0265	— .0057
206	1.0333	1.0333	.0000	244	115	1.0309	1.0321	— .0012
207	1.0350	1.0360	— .0010	820	770	1.0268	1.0283	— .0015
208	1.0300	1.0283	+ .0017	180	260	1.0282	1.0257	+ .0025
209	1.0293	1.0340	— .0047	20	725	1.0291	1.0267	+ .0024
210	1.0348	1.0350	— .0002	228	301	1.0325	1.0320	+ .0005
211	1.0347	1.0309	+ .0038	...	...	.....	.....	.....
212	1.0338	1.0309	+ .0029	...	...	.....	.....	.....
213	1.0252	1.0320	— .0068	...	...	.....	.....	.....
214	1.0345	1.0335	+ .0010	40	11	1.0341	1.0334	+ .0007
215	1.0327	1.0340	— .0013	192	13	1.0308	1.0339	— .0031
216	1.0323	1.0328	— .0005	376	396	1.0285	1.0288	— .0003
217	1.0293	1.0295	— .0002	138	372	1.0279	1.0258	+ .0021
218	1.0338	1.0361	— .0023	...	...	.....	.....	.....
219	1.0330	1.0331	— .0001	...	...	.....	.....	.....
220	1.0321	1.0305	+ .0016	...	...	.....	.....	.....
Hanging								
50	1.0374	1.0331	+ .0043	...	...	.....	.....	.....
51	1.0313	1.0306	+ .0007	23	24	1.0311	1.0304	+ .0007
226	1.0283	1.0383	— .0100	360	1000+	1.0247	1.0283	— .0036
227	1.0440	1.0375	+ .0025	1000+	175	1.0300	1.0357	— .0057
228	1.0320	1.0340	— .0020	238	357	1.0296	1.0304	— .0008
229	1.0329	1.0358	— .0039	52	40	1.0324	1.0354	— .0030
230	1.0355	1.0373	— .0018	500	298	1.0305	1.0343	— .0038
231	1.0368	1.0362	+ .0006	293	330	1.0339	1.0329	+ .0010
232	1.0388	1.0388	.0000	214	70	1.0367	1.0381	— .0014
233	1.0370	1.0395	— .0025	80	20	1.0362	1.0393	— .0031
234	1.0338	1.0351	— .0013	...	...	.....	.....	.....
235	1.0325	1.0435	— .0110	...	...	.....	.....	.....
236	1.0350	1.0350	.0000	46	39	1.0345	1.0346	— .0001
Brain Injuries								
80	1.0300	1.0290	+ .0010	...	...	.....	.....	.....
241	1.0335	1.0363	— .0028	95	205	1.0325	1.0342	— .0017
242	1.0306	1.0306	.0000	...	...	.....	.....	.....
243	1.0312	1.0337	— .0025	...	...	.....	.....	.....
244	1.0260	1.0284	— .0024	...	...	.....	.....	.....
245	1.0339	1.0397	— .0058	...	...	.....	.....	.....
246	1.0382	1.0400	— .0018	...	...	1.0338	1.0368	— .0030
247	1.0280	1.0285	— .0005	37	60	1.0378	1.0394	— .0016
248	1.0286	1.0327	— .0041	280	510	1.0252	1.0234	+ .0018
249	1.0345	1.0350	— .0005	...	...	1.0244	1.0248	— .0004
250	1.0274	1.0278	— .0004	312	465	1.0314	1.0303	+ .0011
251	1.0354	1.0369	— .0015	...	...	.....	.....	.....
252	1.0357	1.0357	.0000	...	...	.....	.....	.....
253	1.0270	1.0247	+ .0023	...	...	.....	.....	.....
254	1.0304	1.0310	— .0006	170	293	1.0287	1.0281	+ .0006
Electrocution								
60	1.0377	1.0357	+ .0020	54	23	1.0372	1.0355	+ .0017
61	1.0337	1.0308	+ .0029	20	30	1.0335	1.0305	+ .0030
260	1.0328	1.0295	+ .0033	100	82	1.0318	1.0287	+ .0031
261	1.0363	1.0343	+ .0020	...	...	.....	.....	.....
262	1.0359	1.0388	— .0029	...	...	.....	.....	.....
Suffocation								
70	1.0365	1.0363	+ .0002	168	103	1.0348	1.0353	— .0005
75	1.0339	1.0336	+ .0003	77	25	1.0331	1.0333	— .0002
270	1.0357	1.0382	— .0025	...	...	1.0358	1.0372	— .0021
271	1.0420	1.0366	+ .0054	...	...	.....	.....	.....
272	1.0346	1.0337	+ .0009	...	...	.....	.....	.....
273	1.0376	1.0347	+ .0029	78	18	1.0368	1.0345	+ .0023
274	1.0293	1.0334	— .0041	12	286	1.0292	1.0305	— .0013
275	1.0333	1.0320	+ .0013	...	...	.....	.....	.....
Gas Asphyxiation								
276	1.0352	1.0410	— .0058	82	48	1.0344	1.0405	— .0061
277	1.0315	1.0340	— .0025	...	...	.....	.....	.....
278	1.0296	1.0300	— .0004	...	...	.....	.....	.....
279	1.0296	1.0305	— .0009	43	44	1.0292	1.0301	— .0009
Poisoning								
281	1.0320	1.0350	— .0030	...	...	.....	.....	.....
282	1.0257	1.0250	+ .0007	86	72	1.0248	1.0243	+ .0005
283	1.0300	1.0342	— .0042	448	243	1.0255	1.0318	— .0063
284	1.0273	1.0288	— .0015	...	...	.....	.....	.....
Miscellaneous								
286	1.0368	1.0335	+ .0028	...	117	.....	1.0323	.....
287	1.0345	1.0353	— .0008	...	...	.....	.....	.....
288	1.0294	1.0304	— .0010	11	21	1.0293	1.0302	— .0009
289	1.0338	1.0362	— .0024	...	...	.....	.....	.....
290	1.0261	1.0236	+ .0025	...	...	.....	.....	.....
292	1.0308	1.0308	— .0005	570	165	1.0246	1.0291	— .0045
293	1.0282	1.0304	— .0022	...	...	.....	.....	.....
294	1.0294	1.0288	+ .0006	...	...	.....	.....	.....

* L indicates left atrium; R, right atrium.

atrial plasma and right atrial plasma. If this value, left-right, was negative, it was concluded that drowning had occurred. If drowning had not been the cause of death, a positive value was obtained. As indicated in Table 1, all the drowning cases first reported [1] continue to show negative values when corrections are applied for the hemoglobin present in the plasma. However, as shown in Table 2, when a similar correction is applied to the 10 controls reported in the first paper, 3 of these give values indicative of drowning. Of the remaining seven, hemoglobin values were available in only four cases. Upon application of the corrections to these four, three gave more positive values for the specific gravity difference than had been previously calculated.

Of the newly considered cases (those numbered above 100) all the drownings in Table 1 show a negative specific gravity difference after correction for hemoglobin. However, such a correction is not necessary in all instances. If a negative difference is found and the left atrial plasma exhibits more hemolysis than that of the right atrium, correction for hemoglobin would only make the difference increasingly negative and therefore is not deemed essential for diagnostic purposes. When a very low negative value, such as that shown in Case 105, is obtained, it is deemed essential to have accurate hemoglobin values for the two plasma samples.

Case 124 represents that of a young boy who became exhausted while swimming and whose body was subsequently recovered from the harbor. The direct specific gravity determinations indicate a nondrowning, with a low positive value of + 0.0004. Upon application of the correction for hemoglobin, this difference becomes —0.0004.

Of the 80 control cases listed in Table 2, corrections for hemoglobin were made in 40. Twelve of these resulted in a change in the algebraic sign, with 6 changing from negative to positive and 6 from zero or positive to negative. Thus, 45 of the control cases remain with negative specific gravity differences even after correction. Hence a negative value, per se, is not conclusive indication of a drowning death, but the finding of a positive difference after correction for any hemoglobin released by hemolysis would definitely prove nondrowning.

It is probable that the lowered plasma specific gravity of the left atrial blood in drowning deaths is due, in varying degree, to hemodilution by water in which the drowning occurs. However, it is a matter of speculation to explain the differences between the specific gravities of the plasma from the two sides of the heart in the control or nondrowning deaths. We have found no absolute correlation between the magnitude of this difference and age, sex, color, lung-heart weight ratios, or postmortem interval. We believe that the observed differences indicate some active process, whether agonal or postmortem, taking place in the body which may in some way be related to the pulmonary edema so frequently observed at autopsy. This factor is the subject of further investigation.

SUMMARY AND CONCLUSIONS

Specific gravity values of plasma from the left and right atria of 80 drowning deaths and 80 control deaths selected at random are reported.

In the use of the specific gravity values in evaluating the possibility of drowning, correction of the observed specific gravity values must be made for the contribution to them of hemoglobin resulting from hemolysis. The exception to this is in cases in which the left atrial plasma obviously contains more hemoglobin than does the right heart plasma and the algebraic difference of left minus right specific gravity is a negative value. Likewise, no correction is needed if the difference is a positive value and the right heart plasma shows more hemolysis than the left.

Negative specific gravity differences after correction for hemoglobin may be obtained in either drowning or nondrowning deaths.

In all the 80 drowning deaths reported, only negative differences were obtained.

Hence, if a positive value is obtained for specific gravity difference after correction for hemoglobin, it is concluded that death was not caused by drowning.

Dr. Russell S. Fisher, Dr. William V. Lovitt, and Spencer R. Watts assisted in this study.

For Reprints and Archives:

REFERENCES

1. Durlacher, S. H.; Freimuth, H. C., and Swann, H. E., Jr.: Blood Changes in Man Following Death Due to Drowning, A. M. A. Arch. Path. **56**:454-461, 1953.

2. Gettler, A. O.: Method for the Determination of Death by Drowning, J. A. M. A. **77**:1650, 1921.

3. Bing, F. C., and Baker, R. W.: Determination of Hemoglobin in Minute Amounts of Blood by Wu's Method, J. Biol. Chem. **92**:589, 1931.

4. Barbour, H. G., and Hamilton, W. F.: Falling Drop Method for Determining Specific Gravity, J. Biol. Chem. **69**:625, 1926.

5. Phillips, R. A.; Van Slyke, D. D.; Hamilton, P. B.; Dole, U. P.; Emmerson, K., Jr., and Archibald, R. M.: Measurement of Specific Gravities of Whole Blood and Plasma by Standard Copper Sulfate Solutions, J. Biol. Chem. **183**:305, 1950.

News and Comment

ANNOUNCEMENTS

Gastrointestinal Cancer Conference.—The Sixth National Gastrointestinal Cancer Conference, sponsored by the gastrointestinal cancer committee of the National Advisory Cancer Council, National Cancer Institute, Public Health Service, will be held on April 4 and 5, 1955. With the New York Cancer Society acting as host, the conference will meet in Hosack Hall, at the New York Academy of Medicine, 5th Ave. and 103d St., New York. The program will emphasize clinical aspects of gastrointestinal cancer. All interested scientists are invited. Dr. Morris K. Barrett of the National Cancer Institute, Bethesda 14, Md., is executive secretary of the gastrointestinal cancer committee. Dr. George T. Pack, New York, is chairman. Correspondence should be addressed to Dr. Barrett.

International Congress of Neuropathology.—The Second International Congress of Neuropathology will be held at the Royal College of Surgeons, Lincoln's Inn Fields, London, on Sept. 12-17, 1955. This Congress is open to all interested persons on payment of the registration fee of £5. Program details and information in general may be obtained by writing to Dr. W. H. McMenemey, Secretary-Treasurer, Maida Vale Hospital for Nervous Diseases, London, W.9.

PERSONAL

Deaths.—Dr. Timothy Leary, Professor Emeritus of Pathology at Tufts College Medical School, died on Nov. 16, 1954, in Medford, Mass., at the age of 84. Dr. Leary served as Medical Examiner in Suffolk County for many years and was particularly well known for his studies on the etiology of atherosclerosis.

Dr. Anna W. Williams, bacteriologist in the New York Department of Health and co-author with Dr. W. H. Park of the well-known textbook of bacteriology, Pathogenic Microorganisms, died in Westwood, N. J., on Nov. 20, 1954, at the age of 91.

Appointments.—Dr. John R. Schenken has been appointed Chairman of the Department of Pathology of the University of Nebraska College of Medicine, at Omaha. Dr. Schenken is also President of the American Society of Clinical Pathologists and of the International Congress of Clinical Pathologists.

Dr. Russell Von Milliser has been made Professor of Pathology in the Chicago Medical School.

Dr. Harry Goldblatt has been appointed Professor of Experimental Pathology in Western Reserve School of Medicine.

Dr. Dale Rex Coman has been appointed chairman of the Department of Pathology at the University of Pennsylvania School of Medicine.

SPONTANEOUS LONGITUDINAL CLEAVAGE OF THE WALL OF THE AORTA

FREDERICK C. BAUER Jr., M.D., and EDWIN F. HIRSCH, M.D., Chicago

THE SPONTANEOUS longitudinal cleavage of the wall of the aorta into an inner and an outer layer is not emphasized in most discussions of the pathogenesis of dissecting aortic aneurysms. Such a cleavage of the wall, prior to the actual hemorrhage, affords a tissue factor favoring the subsequent rupture of the inner tube (or layer) and the escape of blood into a preformed crevice. The extravasation of the blood following the rupture of the inner tube could then further separate the two layers along a plane of least resistance. Only a few isolated reports of such spontaneous longitudinal cleavage of the aorta have been published.

Babes and Mironescu in 1910 [1] described a dissecting aneurysm of the aorta that extended from the innominate artery to the superior mesenteric artery. Below the superior mesenteric artery the aortic wall was actually split longitudinally, or could be split with slight tension. There was no hemorrhage into these tissues. Whitman and Stein in 1924 [2] observed in an embalmed body an aorta with a crevice in the outer part of the media beginning at the base of the heart and extending to within 10 cm. of the iliac bifurcation. The crevice could be extended with little tension and contained a clear, straw-colored fluid regarded as lymph. In summarizing the pathologic changes found in the aorta with dissecting aneurysms, Gore and Seiwert * in 1952 and 1953 commented on the unusual friability of the aortic wall and noted that occasionally a medial defect is obvious whereby longitudinal cleavage or splitting of the aortic wall occurs with slight manipulation. In one aorta (AFIP Acc. No. 51691) described by Gore [5] the media could be separated into an outer and an inner portion throughout its length to the level of bifurcation into the iliac arteries, although the actual intramural hemorrhage did not extend beyond the arch. Indeed, this potential cleavage plane in the outer portion of the media of the aorta and of peripheral arteries is noticed frequently by vascular surgeons during embolectomy and thromboembolectomy procedures,† but this probably is not the same process.

The following report has interest because the clinical symptoms immediately before the death of the patient suggested a diagnosis of dissecting aneurysm of the aorta. However, the necropsy disclosed a longitudinal cleavage of the wall of the aorta without intramural hemorrhage.

REPORT OF A CASE

A salesman, aged 45 years, was brought to the emergency room of St. Luke's Hospital in coma on Jan. 11, 1954, at 5:25 p. m. Death occurred at 7:10 p. m. the same day.

According to information obtained from the police, the man suddenly became sick while driving his car and entered a nearby tavern for help. While there, he collapsed and was brought to the hospital. On arrival the patient appeared to be in severe shock. He did not respond, and his skin was cold, clammy, and cyanotic. Neither acetone nor alcohol was detected on his breath. There were no signs of traumatic injury. Cardiac examination revealed no thrills. The apex of the heart appeared to be approximately 1 cm. to the left of the midclavicular line. The heart sounds were loud and clear, and there was a split aortic second sound, louder than the pulmonic second sound. A Grade II to III harsh systolic murmur was heard over the precordium. The rhythm was regular, and the apical rate was 120 to 130. Coarse, moist rales were heard throughout the left lung. There were a few moist rales in the base of the right lung. The abdomen was soft and had normal bowel tones. The liver, spleen, kidneys, and other masses were not palpable. The lower extremities appeared to have equal color and skin temperature. The femoral, dorsalis pedis, and

From the Henry Baird Favill Laboratory of St. Luke's Hospital.

* References 3 through 5.

† Julian, O. C.: Personal communication to the authors.

posterior tibial pulses were full and equal bilaterally. However, in the upper extremities there was an increase in coldness and cyanosis of the right hand and arm as compared with the left hand and arm. The right radial antecubital and upper brachial arterial pulses were absent. In the left arm, both these pulses were present. The blood pressure in the left arm was 120/60, and in the right arm it was 0. Old bilateral linear surgical scars extended from the lateral chest wall to the flanks (sympathectomy for hypertension at another hospital). The patient's condition became worse, and he died approximately two hours after arrival. The clinical diagnosis was dissecting (aortic) aneurysm.‡

Photograph illustrating the longitudinal cleavage of the wall of the aorta. The inner stripped-up layer of the intima and the inner two-thirds of the media are held away from the outer one-third of the media and adventitia by pins at one end of each segment in order to demonstrate the cleavage plane.

Necropsy disclosed a cleavage of the wall of the aorta, so that when it was opened lengthwise an outer layer, composed of the adventitia and the outer tissues of the media, was separated as a sheet, or membrane, from the inner, remaining portion of the aorta. This cleavage of the aortic wall began in the root of the aorta at the level of the ostium of the coronary arteries and extended to its bifurcation into the common iliac arteries. The cleavage was on the left side and in front except in the arch, where it involved nearly the entire circumference of the aorta. It extended into the innominate artery and into the left renal artery. The tissue space formed by the cleavage did not contain blood, nor was there a tear in the wall of the inner tube. Atherosclerosis of the lining of the aorta was moderate, with some ulceration and calcification. After fixation in a formaldehyde solution and with 10 cm. of aorta attached, the heart weighed 670 gm. The myocardium of the left ventricle was 2 cm. thick. Each kidney weighed 110 gm., and there was a small hemorrhage into the perirenal fat of the left kidney and into the left adrenal gland. There was no massive hemorrhage with blood clots or a break in the intima of the left renal artery. The edematous and hyperemic right lung weighed 1,200 gm., and the similar left lung weighed 870 gm. There were fibrous pleural adhesions on both sides. The liver weighed 1,650 gm. and had moderate passive hyperemia. The brain weighed 1,600 gm. and had no significant changes.

Microscopic examination of the aorta was significant in that there were no marked degenerative changes of the media. The cleavage of the wall occurred at about the level of the junction of the middle third and the outer third of the media, and at other levels of the media were small linear tears, or clefts, that appeared as though the tissue fibers had been pulled apart. Occasionally the vasa vasorum in the media were prominently outlined by medium-sized mononuclear cells, and in the adventitia occasionally they were partially surrounded by lymphocytes and other mononuclear cells. Some of the small arterioles had thick muscular walls and hyaline and fibrous tissue thickenings of the intima, with slight narrowing of the lumens. In addition, there were scattered focal linear changes, most numerous in the outer and middle thirds of the media. Here the orderly fibrous structure of the media was gone, and the fibers were interrupted and separated by mononuclear phagocytes. Also, there were changes of atherosclerosis, with thickening of the intima by masses of hyaline and amorphous material with a few scattered foam cells, and in these regions the media was correspondingly narrowed. Sections stained by the Van Gieson method for elastic fibers revealed some fragmentation of the elastic fibers, but this change was slight. Sections of the kidney had changes of nephrosclerosis, with focal fibrous scars and infiltrations by

‡ The history and physical examination were recorded by Dr. D. B. Horsley.

a few lymphocytes and a hyaline thickening of the walls of the arterioles. The lumen of some of the small arteries was almost occluded by concentrically arranged fibrous tissue thickening of the intima. The glomeruli had fibrous thickenings of Bowman's capsule, and a few were completely hyalinized.

COMMENT

Dissecting aneurysms are defined as those in which blood is forced between coats of an artery. Accordingly, intramural hemorrhage is emphasized as the essential mechanism of this disease, and discussions of pathogenesis are often concerned with the origin of the hemorrhage. Occasionally the hemorrhage is attributed to an "initial" intimal rupture or laceration at the site of an atherosclerotic ulcer. However, intramural hemorrhage of the aorta has been described without rupture of the intima. Thus, Tyson [6] reported three patients without intimal laceration who died with hemopericardium. He suggested that weakening of the media and heightened blood pressure caused one or more vasa vasorum to rupture and produce an intramural hematoma. Internal pressure in the aorta would cause the blood to spread through the weakened media. He considered that intimal laceration is secondary to the formation of the dissecting aneurysm in most, if not all, cases. Gore [4] found 23 cases in the literature without intimal rupture and reported one instance [5] (AFIP Acc. No. 95745) of an isolated hemorrhage 12 cm. long splitting the thoracic and abdominal portions of the aortic media without communication with the intima or adventitia. Glendy, Castleman, and White [7] stated that a common etiological factor is medial degeneration. This condition is a focal or fairly diffuse mucoid or hyaline degeneration which results in cyst formation, sometimes called faults. The frequent location of the intimal tear about 1.5 cm. from the aortic valve suggested that this rupture could be the result of repeated effects of abrupt diastolic recoil, which would force down the aortic valve and the first portion of the aorta, along with supporting structures.

Medial degeneration of the wall in dissecting aneurysms of the aorta as a pathogenic factor has recently been emphasized by Gore,[4] who found in patients under 40 years of age that the elastic laminae were involved and associated with basophilic ground substance, often accumulated in cyst-like spaces and compressing residual intact lamellae. In an older group he found focal degeneration of the smooth muscle of the media, manifested by a pallor of the tissues in stained sections because of the absence of muscle cells and nuclei.[3] Elastic tissue stains demonstrated a condensation of the elastic laminae. The residual fibers in an area of degeneration were swollen and vacuolated. Amromin, Schlichter, and Solway [8] studied 12 dissecting aneurysms of the aorta and in 7 observed narrowing of the lumen of the vasa vasorum produced by arteriosclerosis and arteriolosclerosis, with hypertrophy or hyperplasia of the media and intima. They considered that ischemia of the media of the aorta might be the underlying primary factor in the production of medionecrosis.

Clinical observations of our patient and the necropsy findings clearly indicate that the cleavage of the aortic wall occurred ante mortem. The cleavage was longitudinal. It did not involve portions fixed by the branches of the aorta but was complete in the root and arch, where the entire circumference was split. In the thoracic portion separation of the inner and outer layers did not occur posteriorly because of tissue fixation of this portion of the wall of the aorta by the intercostal arteries and other supporting tissues. The cleavage, accordingly, involved the left and anterior portions of the wall of the aorta, as is common in dissecting aneurysms. Microscopic examination of the wall of the aorta revealed some retrogressive changes in portions of the media, with and without cleavage, but these were not marked.

The occurrence of longitudinal cleavage of the wall of the aorta and death without hemorrhage, as recorded in this report, suggest that dissecting aneurysms of the aorta are not necessarily caused by blood under pressure splitting the wall of the aorta. In

our patient cleavage of the wall occurred without hemorrhage. A cause of this spontaneous cleavage can be the combined effects of torsion and distention on the normally curved arch of the aorta with the pulsations of the blood. Thus, the difference in elasticity between the outer, fibrous adventitia, with some of the media, and the inner, more elastic media tissues would create an expansion and contraction of two dissimilar layers of the aorta. The contraction stress of the inner layer (or tube) would thus pull it away from the outer layer (or tube), eventually with separation of the two. In this way the more expansile and less contractile outer layer would be split longitudinally from the less expansile and more contractile inner layer. Fixation of the two layers at certain levels by the branches of the aorta and the difference in the length of two sides of the aorta caused by the curvature of the arch are other factors that would cause the two dissimilar layers to react unequally to pulsation changes in blood pressure and to separate mechanically. Retrogressive changes of the media, hypertension, or other tissue factors could increase the conditions favoring cleavage. After cleavage had begun, reduced support of the inner tube would increase the possibilities of rupture and the escape of blood into a preformed crevice. The actual entrance of blood into the crevice could further separate the two layers along a plane of least resistance.

SUMMARY

A spontaneous nonhemorrhagic longitudinal cleavage of the wall of the aorta is described. Shock with cyanosis, absence of pulse, and loss of measurable blood pressure in the right arm, as compared with the left arm, were signs clinically interpreted as those of a dissecting aneurysm of the aorta in the patient. The pathogenesis of dissecting aneurysm of the aorta is discussed. The suggestion is made that the extravasation of the blood with a dissecting aneurysm of the aorta is a secondary, and not a primary, factor. The spontaneous cleavage of the wall of the aorta is explained by the combined effects of torsion and distention on the arch of the aorta with the pulsations of the blood and by a difference in elasticity between the outer, fibrous adventitia, with some of the media, and the inner, more elastic media tissues. This difference would gradually separate the inner layer (or tube) from the outer layer (or tube). The more expansile and less contractile outer layer would separate longitudinally from the less expansile and more contractile inner layer. Fixation of the aorta by its branches and by attachment posteriorly to the spine, the difference in length of the two sides of the arch of the aorta, retrogressive changes of the media, and hypertension are factors influencing the cleavage planes and their extent. Reduced support of the inner tube would favor rupture, with the escape of blood into a preformed crevice. The extravasation of blood could further separate the two layers along a plane of least resistance.

REFERENCES

1. Babes, V., and Mironescu, T.: Über dissezierende Arteriitis und Aneurysma dissecans, Beitr. path. Anat. **48**:221, 1910.

2. Whitman, R. G., and Stein, H. B.: Contribution to Pathogenesis of Dissecting Aneurysms: Case of Dissecting Mesaortitis (Babes and Mironescu) Without Dissecting Aneurysm, J. M. Res. **44**:579, 1924.

3. Gore, I., and Seiwert, V. J.: Dissecting Aneurysm of the Aorta—Pathologic Aspects: Analysis of 85 Fatal Cases, A. M. A. Arch. Path. **53**: 121, 1952.

4. Gore, I.: Pathogenesis of Dissecting Aneurysm of the Aorta, A. M. A. Arch. Path. **53**:142, 1952.

5. Gore, I.: Dissecting Aneurysms of the Aorta in Persons Under 40 Years of Age, A. M. A. Arch. Path. **55**:1, 1953.

6. Tyson, M. D.: Dissecting Aneurysms, Am. J. Path. **7**:581, 1931.

7. Glendy, R. E.; Castleman, B., and White, P. D.: Dissecting Aneurysm of the Aorta: Clinical and Anatomical Analysis of 19 Cases (13 Acute) with Notes on Differential Diagnosis, Am. Heart J. **13**:129, 1937.

8. Amromin, G. D.; Schlichter, J. G., and Solway, A. J. L.: Medionecrosis of the Aorta, Arch. Path. **46**:380, 1948.

HOMOGENOUS RENAL TRANSPLANTATION IN DOGS

Associated Positive Coombs Test and Anemia

E. E. MUIRHEAD, M.D.
and
M. GROVES, M.T. (A.S.C.P.), Dallas, Texas
With the Assistance of B. Brooks

THE COOMBS, or antiglobulin, test was introduced by Coombs, Mourant, and Race * for the detection of Rh antibodies, which were designated as "incomplete antibodies." Later Coombs discovered that Moreschi [3] had described the principles of the procedure after a series of experimental manipulations. The indirect Coombs test [4] has been particularly useful in detecting various antibodies to specific erythrocytic antigens and in cross matching blood for transfusions.† The direct Coombs test [4] has been both a diagnostic and an investigative procedure in hemolytic disease of the newborn ‡ following certain incompatible blood transfusions § and in the acquired hemolytic anemias of idiopathic and secondary types.‖

Since the original demonstration by Boorman, Dodd, and Loutit,[11] experience has indicated an almost universal encounter of a positive direct Coombs test in the idiopathic form of acquired hemolytic anemia. In the secondary form of acquired hemolytic anemia this test has been reported positive in half the cases.[19] Moreover, it is not uncommon to encounter a positive indirect Coombs test in the acquired hemolytic anemias when the patient's serum is incubated with the erythrocytes from other subjects.[21] Thus, the Coombs test has assumed a central position in the characterization of acquired hemolytic anemia.

The causative factor in the positive direct Coombs test in acquired hemolytic anemia has been eluted from the erythrocytes ¶ and demonstrated to attach itself to the erythrocytes from other persons. Although earlier studies suggested that this elutable factor acted as an "incomplete panagglutinin," since it coated all erythrocytes tested, more recent observations have indicated specificities which appear identical to those of certain Rh antibodies.# These various observations have resulted in the interpretation that the cause of the positive direct Coombs test in the acquired hemolytic anemias is an autoantibody, and this disturbance has been termed "autoimmune hemolytic disease."[26]

The "autoimmune" concept is, naturally, dependent on an antibody response to an antigen. As long as the "antigen" remains unknown in acquired hemolytic anemia, this concept remains inconclusive and can be challenged.* Thus, additional theoretical possibilities concerning the cause of the positive direct Coombs test may be considered, such as the implication of abnormal globulins unrelated to an antigenic stimulus, and an erythrocytic injury causing alterations that produce either receptors for the antiglobulin serum or receptors for nonimmune globulins, which, in turn, combine with the antiglobulin serum.

Among the difficulties besetting an elucidation of the pathogenesis of acquired hemolytic anemia has been the lack of its reproduction in experimental animals. Since the observations of Dameshek and Schwartz,[30] several studies dealing with immune hemoly-

Supported by a grant from the National Heart Institute, U. S. Public Health Service.

From the Department of Pathology, The University of Texas Southwestern Medical School.

* References 1 and 2.
† References 5 and 6.
‡ References 7 through 9.
§ References 6 and 10.
‖ References 11 through 20.
¶ References 22 and 23.
# References 24 and 25.
* References 27 through 29.

tic states of experimental animals have appeared.† All of these studies, however, have involved heteroimmune or isoimmune erythrocytic antibodies and, as a consequence, have not represented autoimmune states. A possible exception is suggested by the preliminary observations of Smith and coworkers [36] dealing with "autoimmunization" of rabbits with their own trypsinized erythrocytes.

In the present communication we wish to describe the development of an anemia associated with a positive direct Coombs test in the dog following homogenous renal transplantation. The term homogenous indicates the transplantation of the kidney of one dog into a second dog by the anastomosis of artery to artery and vein to vein. The resemblance of the phenomenon to acquired hemolytic anemia of man makes it of interest.

MATERIALS AND METHODS

Adult mongrel dogs of either sex were used. Ether anesthesia was used when bilateral nephrectomy was performed, while pentobarbital sodium was used when only one kidney or no kidney was removed prior to the renal transplantation. The kidneys were removed by the posterior approach. The renal transplant was placed in the neck of the recipient dog by anastomosing the renal artery to the carotid artery and the renal vein to the jugular vein. The ureter was brought to the surface through a stab wound. The donor kidney was placed in a subcutaneous pocket in the neck. The entire procedure required 30 to 45 minutes. Within about five minutes after its conclusion urine flowed from the ureter. Since diuresis usually occurred for a few days, the proper replacement of water and salt became necessary.

The anti-canine-serum rabbit serum (Coombs) was prepared in the rabbit by modifications of the directions of Mourant,‡ as follows: Rabbits weighing 2 to 3 kg. were injected with pooled canine serum obtained by mixing about 5 cc. of serum from each of three to five dogs. A course of intravenous injections was given at two- to three-day intervals, beginning with a 0.5 cc. dose and following with five doses of 1 cc. each. The animal was bled 10 days after the last dose. Pooled erythrocytes from normal dogs were used in absorbing the rabbit serum. The serum was then passed through a Seitz filter, and 1 cc. aliquots in small sterile bottles were stored at —20 C. until used.

The direct Coombs test was conducted by washing a 2% suspension of canine erythrocytes three times in abundant saline at room temperature. After the third washing the test tube was turned up and allowed to drain. To the erythrocytes suspended in the residual saline, approximately a 2% suspension, two drops of the canine antiserum (Coombs) were added. The mixture was allowed to stand for 30 minutes at room temperature, after which it was centrifuged for 1 minute at 500 to 1,000 rpm. The button of cells was shaken into the supernatant fluid, and the results were evaluated grossly according to the following schedule: A solid button represented a 4+ result; a few large clumps were considered as 3+; multiple scattered clumps were graded 2+, and a definitely granular suspension with fine clumps was 1+. All grossly detectable degrees of agglutination were checked microscopically, the clumps of erythrocytes being shown to be stable for more than five minutes. A smooth or fairly smooth suspension which microscopically yielded definite but scattered clumps was designated ±.

The indirect Coombs test was conducted by incubating erythrocytes from normal dogs at 37 C. for 30 minutes, in the form of a 2% suspension in the serum of the dogs that had received a renal transplant, then treating the cells as in the direct procedure. A random panel of canine erythrocytes obtained from the same four or five dogs was used throughout any one experiment.

Eluates of erythrocytes were prepared on two occasions by an adaption of the Landsteiner-Miller [37] procedure as follows: Fifteen to 20 cc. of defibrinated blood was centrifuged; the serum was removed, and the erythrocytes were washed four times with abundant saline. A final 50% suspension of erythrocytes in saline was heated to 56 C. for six to eight minutes, while constantly agitated. The saline was then separated while warm. These eluates did not produce direct agglutination when incubated with a panel of erythrocytes. The ability of the eluate to produce agglutination by means of the indirect Coombs procedure was tested by means of a panel of erythrocytes from normal dogs.

The hematocrit reading was obtained by the method of Wintrobe,[38] the results of which were corrected for trapped plasma according to the method of Chaplin and Mollison.[39] The mechanical fragility of the erythrocytes was determined by a modification of the method of Shen, Castle, and Fleming.[40] One cubic centimeter of oxalated blood (5 cc. of blood plus 10 mg. of buffered double oxalate) was placed in a 25 cc. Erlenmeyer flask containing 20 glass beads, each of about 4 mm. diameter. The flask was clamped to a plastic disc 16.5 cm. from the axis and rotated for 90 minutes

† References 31 through 35.

‡ Cited by Race and Sanger,[4] p. 171.

at 30 rpm, at room temperature. The amount of hemoglobin in the supernatant plasma was related to the total hemoglobin concentration of the blood as percent of hemolysis.

In the course of observations dealing with the influence of homogenous renal transplants on the hypertension following bilateral nephrectomy of dogs,[41] it became apparent that the anemia occurring after the nephrectomies[42] was accentuated following the renal transplantation, even though the "uremia" was improved. When blood transfusions were attempted, they were not as effective as expected (Table 1). Moreover, cross matching of blood became difficult, and autoagglutination of erythrocytes was encountered at room temperature. Since these dogs received blood transfusions at the time of the renal transplantation, the possibility that the phenomenon involved transfused or donor's red cells had to be excluded. Subsequently in dogs that either were given no blood transfusions or received their own blood which had been stored in the refrigerator the same phenomenon was observed. Auto-agglutination was truly present in this group (Table 1) and suggested the need to evaluate the Coombs test under these conditions.

In these early observations, as depicted in Table 1, the renal transplant was applied 7 to 10 days after the second nephrectomy, during which interval the animal was maintained by peritoneal irrigation.[43] In the subsequent observations, which constitute the basis for the present report, four groups of dogs were studied: In Group A the animals had a unilateral nephrectomy, followed in about one week by the homogenous renal transplant; in Group B the animals had a bilateral nephrectomy, followed in 24 hours by the homogenous renal transplant; in Group C animals with their kidneys intact received a homogenous renal transplant, which was followed in 24 hours by an acute hemolysis induced by phenylhydrazine (40 mg. per kilogram of body weight, given intravenously as a 2% solution in saline), and in Group D intact animals received the homogenous renal transplant, which was followed in 24 hours by the withdrawal

TABLE 1.—*Blood Changes in Three Dogs* Following Nephrectomy and Homogenous Renal Transplant*

Dog No. Wt., Kg.	Days	Blood Volume (T 1824), Cc.	RBC Volume, Cc.	Hematocrit, %	Blood Transfusion, Cc.
A	0	1,670	417	25	
14.5	0	Second nephrectomy			
	1-31	Peritoneal irrigation			
	10	1,270	338	27	400
	10	Homogenous renal transplant			
	15	1,265	296	23	
	24	1,020	160	15	250
				18†	
	27			14	500
				33†	
	30			17	300
				24†	
	31			21	
B	0	Second nephrectomy			
8.4	1-16	Peritoneal irrigation			
	9	Homogenous renal transplant			
	14	(*a*) Autoagglutination 2+; room temp.			
		(*b*) Suspension of Dog B's RBC in normal dog's serum; room temp.; agglutination 3+			
	16	Died			
C	—6	Removal of 300 cc. of blood; storage at 5 C.			
13	0	Second nephrectomy			
	1-11	Peritoneal irrigation			
	4	Homogenous renal transplant			
	4-8	Return of 300 cc. of own blood			
	10	(*a*) Autoagglutination 1+; room temp.			
		(*b*) Direct Coombs test 1+			
		(*c*) Indirect Coombs test with Dog C's serum; negative for 4 bloods			
		(*d*) Eluate of Dog C's RBC; no agglutination against 4 bloods directly			
		Indirect Coombs test against eluate positive with 3 of 4 bloods			

* Dog A demonstrates the accentuation of the anemia 5 to 10 days after the homogenous renal transplantation and the ineffectiveness of blood transfusions (1,050 cc.) on this anemia. Dog B demonstrates autoagglutination at room temperature after the homogenous renal transplantation in the absence of blood transfusion and, also, agglutination when the washed cells were placed in another dog's serum. Dog C demonstrates autoagglutination and a positive direct Coombs test following the homogenous renal transplant. In addition, an eluate prepared from the Coombs-positive red cells yielded a positive indirect Coombs test with three of four erythrocyte suspensions obtained from four normal dogs.

† Same day, after transfusion.

of the animal's own blood (about 30 cc. per kilogram of body weight), hemolysis of the blood by freezing and thawing, and the reinfusion of the hemolyzed autogenous blood.

The hemolytic state was interposed in Groups C and D because the Coombs test appeared more evident when the transplantation was conducted 7 to seven days later).—Of the four dogs in this group, three developed a positive direct Coombs test of 1+ to 3+ magnitude. The mechanical fragility of the erythrocytes was significantly elevated in two dogs but receded to control levels in 15 to 28 days. At this

TABLE 2 *(Group A).—Results Following Unilateral Nephrectomy Plus Homogenous Renal Transplantation Seven Days Later* *

Dog No.	Days	Direct Coombs Test	Hematocrit, %	Mechanical Fragility, % Hemolyzed	Indirect Coombs Test, RBC				
					1	2	3	4	5
1	0	—	40.0	6.1	..	..	..	..	..
	3	—	34.3	22.0	..	..	..	..	..
	6	±	29.4	31.2	..	..	..	..	..
	9	1+	30.0	42.6	—	—	1+	1+	—
	28	1+	35.0	3.9	..	..	..	..	..
2	0	—	44.0	6.6	..	..	..	..	..
	3	—	36.0	11.3	—	—	—	—	—
	5	3+	39.0	3.3	..	..	..	..	..
	9	Died							
3	6	1+	34.0	25.0	—	—	—	—	—
	8	1+	29.6	15.4	..	1+	..	..	1+
	10	1+	28.0	12.8	—	—	—	1+	—
	15	—	29.5	2.3	—	—	—	—	—
	51	—	40.5	3.7	..	..	..	..	..
4	0	..	45.5	3.8	..	..	..	..	..
	3	—	38.0	2.2	—	—	—	—	—
	5	—	37.0	2.6	—	—	—	—	—
	41	—	33.5	2.3	—	—	—	—	—

* The minus sign indicates negative results; the dots indicate that no tests were performed at that time.

TABLE 3 *(Group B).—Results Following Bilateral Nephrectomy Plus Homogenous Renal Transplantation Twenty-Four Hours Later*

Dog No.	Days	Direct Coombs Test	Hematocrit, %	Mechanical Fragility, % Hemolyzed	Indirect Coombs Test, RBC			
					1	2	3	4
5	0	—	42.0	5.4	..	..	..	..
	4	1+	23.0	9.0	—	1+	1+	1+
	7	—	18.0	32.2	—	1+	3+	—
	12	Died						
6	0	..	44.0	4.8	..	..	..	..
	6	—	21.5	37.6	—	1+	1+	1+
	9	—	23.0	23.5	—	—	—	—
	14	—	24.0	42.0	—	—	—	—
	22	—	32.0	69.0	..	..	..	..
	24	Died						
7	4	—	27.6	30.2	..	..	..	..
	6	—	17.0	60.4	—	—	—	1+
	7	—	13.7	76.0	—	—	—	—
8	0	..	42.0	5.0	..	..	..	..
	5	—	22.7	87.0	—	—	—	—
	11	Died						

10 days after the second nephrectomy, as opposed to the cases in which the transplantation was effected 24 hours after the second nephrectomy. Since by 7 to 10 days after the nephrectomies a hemolytic anemia [42] is evident, it was considered possible that hemolysis or its products might potentiate the phenomenon.

RESULTS

GROUP A (Table 2; unilateral nephrectomy plus homogenous renal transplantation time and beyond, recovery from the anemia was progressing. The indirect Coombs test was positive to the erythrocytes from two of five dogs in one case and to the cells of three of five dogs in another. In one dog the findings were negative except for the development of anemia.

GROUP B (Table 3; bilateral nephrectomy plus homogenous renal transplantation in

24 hours).—The animals in Group B died after two to three weeks, although during most of this time uremia was not prominent, since the transplanted kidney was able to sustain renal function.[44] Only one of the four dogs in this group developed a positive direct Coombs test, and in this case the phenomenon was observed only once. All animals developed a prominent anemia, associated with a markedly elevated mechanical fragility of the erythrocytes. The indirect Coombs test became positive to the erythrocytes from three of four dogs in two dogs and to the cells of one of four dogs in another.

GROUP C (Table 4; homogenous renal transplantation in intact animal plus phenyl-

TABLE 4 *(Group C).—Results Following Homogenous Renal Transplantation of Intact Animal Plus Phenylhydrazine Twenty-Four Hours Later*

Dog No.	Days	Direct Coombs Test	Hemato-crit, %	Mechanical Fragility, % Hemolyzed	Indirect Coombs Test, RBC			
					1	2	3	4
9	0	..	48.0	7.0	..	..	..	..
	4	1+	20.0	41.9	—	1+	1+	1+
	7	1+	17.1	55.0	3+	3+	±	3+
	8	3+	8.0	88.6	3+	2+	2+	3+
10	0	..	44.5	15.5	..	..	..	..
	3	—	22.3	50.1	..	..	..	..
	5	3+	6.0	33.1	..	..	..	..
11	0	..	47.0	11.2	..	..	..	..
	4	—	23.0	35.2	—	—	—	—
	6	—	17.0	84.8	3+	3+	—	3+
	7	1+	....	....	Hem.*	3+	—	3+
	8	1+	8.0	64.7	3+	3+	—	3+

* Hem. = hemolyzed.

TABLE 5 *(Group D).—Results Following Homogenous Renal Transplantation Plus Infusion of Hemolyzed Autogenous Blood Twenty-Four Hours Later*

Dog No.	Days	Direct Coombs Test	Hemato-crit, %	Mechanical Fragility, % Hemolyzed	Indirect Coombs Test, RBC		
					1	2	3
12	0	—	38.2	4.4	—	—	—
	1	—	35.7	5.15	±	—	2+
	2	—	32.9	4.4	..	..	..
	5	—	28.4	16.7	Hem.*	±	±
	8	±	....	9.1	2+	1+	Incomp.†
	12	1+	26.5	6.0	Hem.	Hem.	Incomp.
	20	±	30	8.3	Incomp.	±	Incomp.
13	0	..	44.8	...	..	..	..
	4	—	23.0	...	..	..	..
	6	2+	25.5	...	..	..	..
	7	1+	26.5	...	..	..	..
	10	—	28.5	...	..	..	..
	12	3+	31.5	...	..	..	..
14	0	—	46.0	5.2	..	..	..
	1	..	44.0	9.8	..	..	..
	2	1+	30.9	4.4	..	..	..
	4	±	29.9	13.5	..	..	..
	6	±	22.4	22.4	..	..	..
	8	±	22.3	6.5	..	..	..
	11	1+	22.3	5.1	..	..	..
	14	—	30.5	9.1	..	..	..
	19	—	29.4	25.8	..	..	..
15	0	—	38.0	3.6	..	..	..
	4	—	21.0	38.6	..	..	..
	7	±	23.0	37.3	..	..	..
	8	1+	16.0	...	..	..	..
	26	—	29.3	36.5	..	..	..
	32	—	....	...	..	..	..
	34	—	34.2	14.0	..	..	..
	36	—	34.6	3.2	..	..	..

* Hem. = hemolyzed.
† Incomp. = incompatibility.

hydrazine in 24 hours).—All three of the dogs in Group C developed a positive direct Coombs test within four to seven days. There was a pronounced anemia and a marked elevation of the mechanical fragility of the erythrocytes.§ The indirect Coombs test became positive to the erythrocytes from four of four dogs tested in one animal and to the cells of three of four dogs in another.

Group D (Table 5; homogenous renal transplantation plus an infusion of hemolyzed autogenous blood in 24 hours).—All four dogs in this group developed a positive direct Coombs test. Three animals so studied yielded an elevation of the mechanical fragility of the erythrocytes. Recovery from the anemia was apparent by two weeks. The only animal in this group tested with the indirect Coombs test yielded, at an earlier time, a positive test to the erythrocytes from three normal dogs and, at a later time, either hemolysis or saline agglutination (direct incompatibility) to the same cells.

Summary of Results.—Of the 16 dogs subjected to a homogenous renal transplantation and tested with the anticanine serum, or Coombs serum (Tables 1 through 5), 12 (75%) developed a positive direct Coombs test. In eight cases the gross magnitude of the test consisted of a 1+ reaction. In four cases the gross agglutination varied between 2+ and 3+. The development of the positive direct Coombs test was associated with an accentuation of the anemia. In most cases the mechanical fragility of the erythrocytes became prominently elevated. A positive indirect Coombs test was frequently encountered when the serum of the dog with a transplanted kidney was tested against the erythrocytes obtained from three to five normal dogs.

COMMENT

The time during which the direct Coombs test became positive in the present experiments is of interest. While in 50% of the animals the test became positive in 5 to 7 days, it was positive in 5 to 10 days in 75% of the cases. In two instances the test was positive in four days, and only once was it positive within two days. Thus, the positive direct Coombs test under these circumstances seemed to require an interval of approximately one week for its development.

The interval of about one week represents the time during which deterioration of the functional state of the renal transplant becomes apparent.‖ At this time, also, the transplant frequently reveals gross signs of softening and resorption. Beyond one week the resorption increases, and by two to three weeks the structure frequently decreases in size and ceases its output of urine. It is evident that the positive direct Coombs test appeared at approximately the time when deterioration and resorption of the homogenous renal transplant became overt.

In five dogs studied over a prolonged period of time (19 to 51 days), the direct Coombs test became negative and the manifestations of the anemia and abnormal mechanical fragility of the erythrocytes abated. The negative test was observed between 3 and 18 days after the last positive test, and in three of the five examples it was negative 14 to 26 days after the renal transplantation. Thus, the combination of anemia, increased mechanical fragility of the erythrocytes, and positive direct Coombs test was transient when the period of observation was prolonged. Moreover, the direct Coombs test became negative usually beyond the time of maximal resorption of the renal transplant.

The factor causing the positive direct Coombs test appeared to be elutable from the erythrocytes in the example related in Table 1. The temporal development and recession of the positive direct Coombs test and the associated erythrocytic abnormalities pose the question of whether the factor causing the positive Coombs test is an antibody against the red blood cells of the renal recipient or whether it represents nonimmune globulins or other proteins derived from the

§ Subsequently it was demonstrated that phenylhydrazine alone induces a positive direct Coombs test in the dog. This has been made the subject of a separate report.[45]

‖ References 9, 11, and 12.

resorbing renal transplant and having the ability to attach themselves to the erythrocytes of the dog with a transplant.

A third theoretical possibility in the pathogenesis of the positive direct Coombs test following the renal transplantation must be considered in view of additional observations from this laboratory.[10] In attempting to control the observations of Group C (renal transplant plus hemolysis by phenylhydrazine), we demonstrated that the action of phenylhydrazine by itself evokes a positive direct Coombs test in the dog. For several reasons, outstanding among which was the observation that the test usually became positive within four to seven days, the finding was interpreted as the result of a direct erythrocytic injury. It was considered that such an injury might cause the positive test directly by affecting receptors on the red cells for the anticanine serum or indirectly by affecting receptors for nonimmune globulins, which, in turn, combine with the anticanine serum. In the light of this experience, it is conceivable that the products derived during the resorption of the homogenous renal transplant may be directly injurious to the recipient's erythrocytes and thus evoke the positive direct Coombs test in a manner comparable to the considered action of phenylhydrazine.

These considerations indicate that the data presented herein do not elucidate the cause of the positive direct Coombs test. They merely pose three major questions: 1. Is the positive direct Coombs test due to immune globulins against the donor's kidney which at the same time are capable of attachment onto the recipient's erythrocytes, and thus act like an autoimmune erythrocytic antibody? 2. Is it due to nonimmune globulins or other proteins derived from the resorbing renal transplant which are able to become attached to the recipient's erythrocytes? 3. Or is it due to an erythrocytic injury from products of resorption from the transplanted kidney?

The temporal development of the positive direct Coombs test (within 5 to 10 days) would be in keeping with all three possibilities. The disappearance of the positive test and the concomitant abatement of the anemia and increased erythrocytic fragility within 14 to 26 days constitute distinct weaknesses in the antibody or immune concept. On the other hand, the transient nature of the phenomenon is in keeping with the nonimmune possibilities embodied in the second and third proposals listed above. Work is now in progress attempting to elucidate these possibilities.

The indirect Coombs test applied to the recipient's serum indicated a specificity of the serum, even though only the erythrocytes from a few normal dogs were included in the panel. It was not possible to flush out all of the donor's erythrocytes within the renal transplant. Thus, the recipient's serum activity may have been due to isoimmune erythrocytic antibodies. These antibodies would not ordinarily be considered eligible to attach onto the recipient's erythrocytes. In view of recent indications for the specificity of certain "autoimmune antibodies" in acquired hemolytic anemia,¶ this point cannot be considered certain without additional observations.

The encounter of a positive direct Coombs test in all eight dogs (Tables 1, 4, and 5) having an active hemolytic state in addition to the renal transplant suggests the need for further observations on the possible potentiation of the phenomenon by products of hemolysis. Previously [10] it was demonstrated that hemolysis per se in the test tube or in vivo did not cause a positive direct Coombs test with the Coombs serum used in these experiments.

After the present work was begun, the observations of Simonsen [46] concerning serologic investigations in dogs following renal transplantation came to our attention. In one experiment, 20 to 21 days after the renal transplantation, Simonsen noted autoagglutination and panagglutination when the recipient's erythrocytes were placed in canine serum from different sources. A positive di-

¶ References 13 and 14.

rect Coombs test was noted after transplantation of the spleen in one experiment. After transplantation of kidneys, however, the direct Coombs test was negative when the red cells were washed with saline. It is apparent that Simonsen's investigations indicated changes in the direction of, but not identical with, the ones herein recorded. For reasons that are not clear, but that possibly include products of hemolysis as described above, homogenous renal transplantation in our experience has been attended by a high incidence of positive direct Coombs tests. This finding occurred even though the erythrocytes were thoroughly washed in saline, in contradistinction to the observations of Simonsen.

SUMMARY

The triad of a positive direct Coombs test, anemia, and increased mechanical fragility of the erythrocytes was observed following homogenous renal transplantation in the dog.

The direct Coombs test usually became positive within 5 to 10 days after the transplantation.

In dogs observed over a prolonged interval the triad (positive direct Coombs test, anemia, increased erythrocytic fragility) abated within 14 to 26 days.

The implications of these findings are discussed.

REFERENCES

1. Coombs, R. R. A.; Mourant, A. E., and Race, R. R.: New Test for the Detection of Weak and "Incomplete" Rh Agglutinins, Brit. J. Exper. Path. **26**:255, 1945.

2. Coombs, R. R. A.; Mourant, A. E., and Race, R. R.: Detection of Weak and "Incomplete" Rh Agglutinins: New Test, Lancet **2**:15, 1945.

3. Moreschi, C.: Neue Tatsachen über die Blutkörpchenagglutination, Zentralbl. Bakt. **46**:49, 1908.

4. Race, R. R., and Sanger, R.: Blood Groups in Man, Springfield, Ill., Charles C Thomas, Publisher, 1951.

5. Mollison, P. L.: Blood Transfusion in Clinical Medicine, Springfield, Ill., Charles C Thomas, Publisher, 1952, pp. 222, 240, and 286.

6. Hutcheson, J. B.; Haber, J. M., and Kellner, A.: Hazard of Repeated Blood Transfusion: Hemolytic Reaction Due to Antibodies to the Duffy (Fy^a) Factor, J. A. M. A. **149**:274, 1952.

7. Coombs, R. R. A.; Mourant, A. E.; and Race, R. R.: In-Vivo Isosensitisation of Red Cells in Babies with Hæmolytic Disease, Lancet **1**:264, 1946.

8. Hill, J. M., and Haberman, S.: Demonstration of Rh Antibodies in the Newborn and Further Evidence of the Pathogenesis of Erythroblastosis, J. Lab. & Clin. Med. **31**:1053, 1946.

9. Levine, P.: Recent Developments in Iso-Immunization by the Rh Factor, Am. J. Obst. & Gynec. **49**:810, 1945.

10. Muirhead, E. E., and Groves, M.: Problems in Blood Transfusion: Minor Incompatible Blood Transfusions and Acquired Hemolytic Anemia, Texas J. Med. **50**:465, 1954.

11. Boorman, K. E.; Dodd, B. E., and Loutit, J. F.: Hæmolytic Icterus (Acholuric Jaundice): Congenital and Acquired, Lancet **1**:812, 1946.

12. Sturgeon, P.: New Antibody in Serum of Patients with Acquired Hemolytic Anemia, Science **106**:293, 1947.

13. Evans, R. S.; Duane, R. T., and Behrendt, V.: Demonstration of Antibodies in Acquired Hemolytic Anemia with Anti-Human Globulin Serum, Proc. Soc. Exper. Biol. & Med. **64**:372, 1947.

14. Evans, R. S., and Duane, R. T.: Acquired Hemolytic Anemia: I. Relation of Erythrocyte Antibody Production to Activity of the Disease; II. Significance of Thrombocytopenia and Leukopenia, Blood **4**:1196, 1949.

15. Dacie, J. V., and de Gruchy, G. C.: Auto-Antibodies in Acquired Hæmolytic Anæmia, J. Clin. Path. **4**:253, 1951.

16. Dameshek, W.: Acquired Hemolytic Anemia: Physiopathology with Particular Reference to Auto-Immunization and Therapy, in Moore, Carl V., Editor-in-chief: Proceedings of the Third International Congress of the International Society of Hematology, Cambridge, England, Aug. 21-25, 1950, New York, Grune & Stratton, Inc., 1951, p. 120.

17. Wiener, A. S.; Samwick, A. A.; Morrison, M., and Loewe, L.: Acquired Hemolytic Anemia, Am. J. Clin. Path. **22**:301, 1952.

18. Rosenfield, R. E.; Vogel, P., and Rosenthal, N.: Antiglobulin Test: Technic and Practical Application, Am. J. Clin. Path. **21**:301, 1951.

19. Davidsohn, I., and Oyamada, A.: Specificity of Auto-Antibodies in Hemolytic Anemia, Am. J. Clin. Path. **23**:101, 1953.

20. Dacie, J. V.: Acquired Hemolytic Anemia with Special Reference to the Antiglobulin (Coombs') Reaction, Blood **8**:813, 1953.

21. Young, L. E.; Miller, G., and Christian, R. M.: Clinical and Laboratory Observations on Autoimmune Hemolytic Disease, Ann. Int. Med. **35**:507, 1951.

22. Kidd, P.: Elution of an Incomplete Type of Antibody from the Erythrocytes in Acquired Hæmolytic Anæmia, J. Clin. Path. **2**:103, 1949.

23. Komninos, Z. D., and Rosenthal, M. C.: Studies on Antibodies Eluted from the Red Cells in Autoimmune Hemolytic Anemia, J. Lab. & Clin. Med. **41**:887, 1953.

24. Weiner, W.; Battey, D. A.; Cleghorn, T. E.; Marson, F. G. W., and Meynell, M. J.: Serological Findings in a Case of Haemolytic Anaemia, with Some General Observations on the Pathogenesis of This Syndrome, Brit. M. J. **2**:125, 1953.

25. Dacie, J. V., and Cutbush, M.: Specificity of Auto-Antibodies in Acquired Hæmolytic Anæmia, J. Clin. Path. **7**:18, 1954.

26. Young, L. E., and Miller, G.: Long-Term Picture in Autoimmune Hemolytic Disease, Tr. A. Am. Physicians **66**:190, 1953.

27. Wagley, P. F.; Shen, S. C.; Gardner, F. H., and Castle, W. B.: Studies on the Destruction of Red Blood Cells: VI. The Spleen as a Source of a Substance Causing Agglutination of Red Blood Cells of Certain Patients with Acquired Hemolytic Jaundice by an Antihuman Serum Rabbit Serum (Coombs' Serum), J. Lab. & Clin. Med. **33**:1197, 1948.

28. Castle, W. B., in Beeson, P. B., Editor: Year Book of Medicine, Chicago, Year Book Publishers, Inc., 1950, p. 277.

29. Castle, W. B., in Beeson, P. B., Editor: Year Book of Medicine, Chicago, Year Book Publishers, Inc., 1951, p. 360.

30. Dameshek, W., and Schwartz, S. O.: Hemolysis as the Cause of Clinical and Experimental Hemolytic Anemias, with Particular Reference to Nature of Spherocytosis and Increased Fragility, Am. J. M. Sc. **196**:769, 1938.

31. Tigertt, W. D., and Duncan, C. N.: Erythrocyte Morphology in Experimental Hemolytic Anemia as Induced by Specific Hemolysin, Am. J. M. Sc. **200**:173, 1940.

32. Christian, R. M.; Ervin, D. M., and Young, L. E.: Observations on the In-Vitro Behavior of Dog Isoantibodies, J. Immunol. **66**:37, 1951.

33. Christian, R. M.; Stewart, W. B.; Yuile, C. L.; Ervin, D. M., and Young, L. E.: Limitation of Hemolysis in Experimental Transfusion Reactions Related to Depletion of Complement and Isoantibody in the Recipient: Observations on Dogs Given Successive Transfusions of Incompatible Red Cells Tagged with Radioactive Iron, Blood **6**:142, 1951.

34. Young, L. E.; Christian, R. M.; Ervin, D. M.; Davis, R. W.; O'Brien, W. A.; Swisher, S. N., and Yuile, C. L.: Hemolytic Disease in Newborn Dogs, Blood **6**:291, 1951

35. Chute, R. N., and Sommers, S. C.: Hemolytic Disease and Polycythemia in Parabiosis Intoxication, Blood **7**:1005, 1952.

36. Smith, J. W.; Dodd, M. C.; Wright, C.-S., and Bunner, A. E.: Iso-Immunization of Rabbits with Trypsinized Erythrocytes, Fed. Proc. **13**: 512, 1954.

37. Landsteiner, K., and Miller, C. P., Jr.: Serological Studies on the Blood of the Primates: II. Blood Groups in Anthropoid Apes, J. Exper. Med. **42**:853, 1925.

38. Wintrobe, M. M.: Simple and Accurate Hematocrit, J. Lab. & Clin. Med. **15**:287, 1929.

39. Chaplin, H., Jr., and Mollison, P. L.: Correction for Plasma Trapped in the Red Cell Column of the Hematocrit, Blood **7**:1227, 1952.

40. Shen, S. C.; Castle, W. B., and Fleming, F. M.: Experimental and Clinical Observations on Increased Mechanical Fragility of Erythrocytes, Science **100**:387, 1944.

41. Muirhead, E. E.: Homogenous Renal Transplants After Bilateral Nephrectomy; abstracted, Am. J. Med. **13**:649, 1952.

42. Muirhead, E. E.; Jones, F.; Stirman, J. A., and Lesch, W.: Hemolytic Anemia of the Dog Following Bilateral Nephrectomy, Am. J. Physiol. **173**:371, 1953.

43. Muirhead, E. E.; Stirman, J. A.; Jones F.; Lesch, W.; Burns, M., and Fogelman, M. J.: Cardiovascular Lesions Following Bilateral Nephrectomy of Dog: Role of Hypertension and Other Factors on Pathogenesis, A. M. A. Arch. Int. Med. **91**:250, 1953.

44. Muirhead, E. E.: Acute Renal Failure, Basic Science Notes, Army Medical Dept. Research and Graduate School, 1954, p. 909.

45. Muirhead, E. E.; Groves, M., and Bryan, S.: Positive Direct Coombs Test Induced by Phenylhydrazine, J. Clin. Invest. **33**:1700, 1954.

46. Simonsen, M.: Biological Incompatibility in Kidney Transplantation in Dogs: II. Serological Investigations, Acta path. et microbiol. scandinav. **32**:36, 1953.

EFFECTS OF ETHIONINE INJECTIONS ON PREGNANT RATS AND THEIR OFFSPRING

C. M. LEE Jr., M.D.; J. T. WISEMAN, B.A.; S. A. KAPLAN, M.D., and J. WARKANY, M.D., Cincinnati

FARBER and Popper,[4] in 1950, observed the loss of cytoplasmic basophilia of the pancreatic acinar cells and diffuse pancreatitis in rats injected intraperitoneally with ethionine. They found that the development of pancreatitis could be prevented by simultaneous administration of methionine. In the same year, Goldberg, Chaikoff, and Dodge [7] described complete obliteration of the acinar pancreatic tissue, accompanied by fibrous proliferation, after injections of DL-ethionine. These findings, which were soon confirmed by other investigators,* suggested the possibility of producing fibrosis of the pancreas in the young of pregnant rats by the injection of DL-ethionine during the gestational period. It was hoped that the administration of ethionine during or soon after development of the fetal pancreas would produce disturbances comparable to those seen in newborn children with cystic fibrosis of the pancreas and, possibly, with meconium ileus. Although this objective was not achieved, the experiments did yield interesting results, which deserve to be recorded.

METHODS

Sixty-four pregnant albino rats of a commercial strain were injected intraperitoneally with a solution of 2.5% DL-ethionine † in distilled water. Attempts were made to establish a dosage and injection schedule which would damage the maternal pancreas, yet permit survival of both mother and fetus to the end of gestation. Individual females were injected daily with doses of from 25 to 75 mg. of ethionine over periods ranging from 1 to 16 days in a single pregnancy. The duration of the injection period depended upon the response of the female; if massive vaginal bleeding was observed and resorption threatened, injections were discontinued completely, or for as long as resorption seemed imminent. This commonly occurred around the 15th day of pregnancy. The massive vaginal bleeding characteristic of resorption can easily be distinguished from the normal blood sign, which is a slight discoloration of the vaginal smear, occurring between the 12th and the 14th day of gestation. If the pregnancy continued, injections were resumed and given to the end of gestation. On the 21st or 22d day of pregnancy the mothers were killed and the young removed by Caesarean section.

The 64 female rats injected in this manner weighed 190 to 250 gm. at the beginning of the experiment. They were fed a diet adequate for growth and reproduction. According to treatment and results, they can be divided into three groups. The six females in Group I received totals of 200 mg. of ethionine or less during the entire pregnancy.

In Group II, 41 rats were given total injections ranging from 250 to 850 mg. of ethionine per pregnancy. The most frequent mode of administration in this group was daily injection of 50 mg. from the 9th to the 21st day, amounting to a total of 650 mg. (13 rats). But, as stated before, in many cases modifications were necessitated by threatened resorption, and the total dose was reduced to from 250 to 600 mg. (22 rats). Six of the rats were given somewhat larger doses, totaling from 675 to 850 mg. per pregnancy, because ethionine had been administered before the ninth day. The weights of the females increased normally between conception and the first injection; during the experimental period either the weight gain was less than that of the controls (which gained an average of 60 gm. between the 10th and the 22d day of gestation) or there was a loss in weight.

Group III is comprised of those females which resorbed their young completely once or several times. When resorption was certain, the injections were interrupted and the females remated. During the ensuing pregnancy injections were resumed. The 17 females in this group underwent a total of 37

From the Departments of Surgery and Pediatrics and the Children's Hospital Research Foundation, University of Cincinnati College of Medicine.

This study was supported in part by grants from the Nutrition Foundation, Inc., New York; the National Vitamin Foundation, Inc., and the National Institutes of Health, Metabolism and Nutrition Study Section, Public Health Service (A-427).

* References 1, 2, 6, 9, 11, 16, and 17.

† Nutritional Biochemicals Corporation, Cleveland.

pregnancies during the course of the experiment. The total doses administered to each individual rat varied from 750 to 1,350 mg., during two to four pregnancies.

Pregnancy was verified by the discovery of sperm in the vaginal smear. The day on which sperm were found was designated as the first day of pregnancy. Resorption was determined by loss of weight, massive vaginal bleeding, or the appearance of estrous cells in the vaginal smear.

Histologic sections were prepared from the maternal pancreas, liver, and kidney in each adult rat. In a few animals the adrenal glands were also studied histologically. The young were formalin-fixed and subjected to serial section of the entire body in representative samples from each litter.

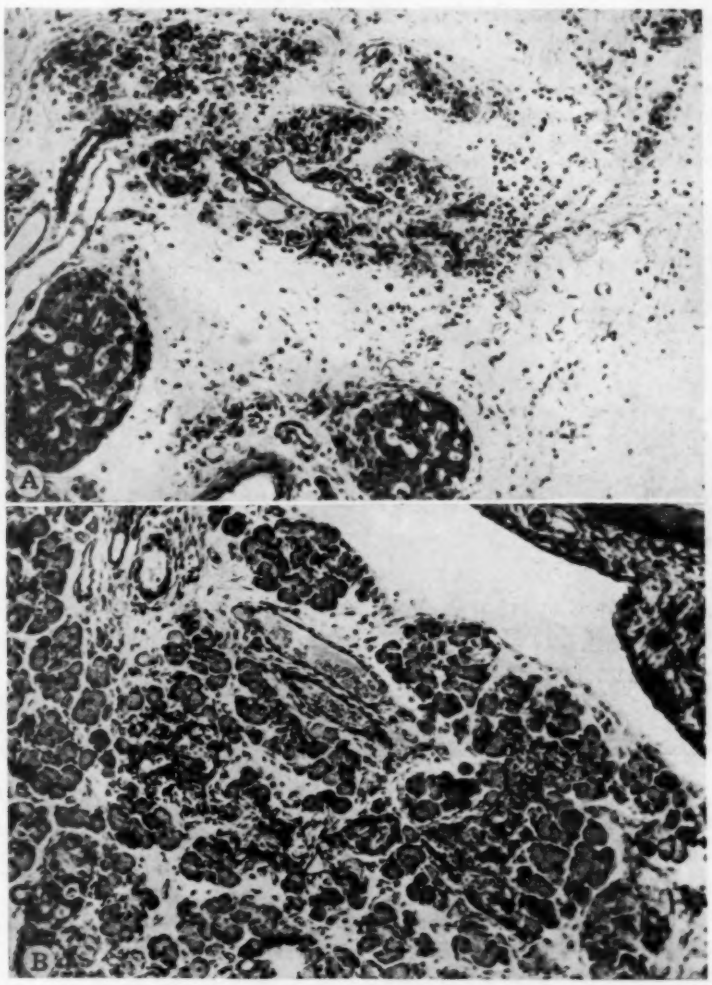


Fig. 1.—*A*, photomicrograph (reduced ¼ from mag. × 160) of pancreas of adult female rat which received a total of 600 mg. of DL-ethionine from the 10th to the 21st day of pregnancy. This section shows almost complete loss of pancreatic exocrine tissue. Note slight mononuclear infiltration, scattered residual atrophic acini, and normal islets; *B*, photomicrograph of section from pancreas of one of the young from the mother whose pancreas is illustrated in *A*. The pancreas of the young is essentially normal. Reduced ¼ from mag. × 160.

No pathologic changes were noted in any of the adrenal glands studied. Damage to the liver ranged from none to very mild toxic hepatitis, and to the kidneys, from none to moderately severe tubular

damage. The glomeruli appeared normal in all cases. No effort was made to study the renal and hepatic changes in detail, since these have been described by others and were not pertinent to the primary purpose of this study.

Each maternal and fetal pancreas was subjected to closer scrutiny. Bensley's acid fuchsin, methyl green, and modified aniline blue stains for pancreas were found to be too variable in performance to permit satisfactory comparisons, so that hematoxylin and eosin sections were utilized for microscopic study. A rather constant feature of the degenerative changes to be described below was an increasingly basophilic stain of acinar cells, apparently due to loss of secretory granules and corresponding roughly to the severity of the damage. The changes were classified in three categories, as follows:

1. *Mild Toxic Pancreatitis.*—This was characterized by mild interstitial edema and cloudy swelling of the acinar cells. Impairment of clear visualization of the zymogen granules was consistently present but was found to be difficult of qualitative evaluation.

2. *Moderate Degenerative Pancreatitis.*—Here the interstitial edema was more pronounced, and mononuclear cell infiltration was frequently striking. The architecture of the acini was markedly disturbed, with degenerative changes in both cytoplasmic and nuclear structure. Loss of zymogen granules and decrease in average acinar cell size were followed by disruption of acini and eventual disappearance of both acini and rounded isolated acinar cells.

3. *Severe Degenerative Pancreatitis.*—This condition was characterized by advanced degenerative changes and total destruction of much of the exocrine tissue of the pancreas. Destruction was never complete in the mothers killed after a single pregnancy (600 to 850 mg. of ethionine). In two of the rats subjected to three pregnancies, with correspondingly large total doses, no recognizable pancreatic tissue could be found. In all others recognizable, but scattered, groups of acini could be seen, uniformly in an advanced stage of degeneration. In all these instances the undamaged islets of Langerhans stood out prominently in an amorphous edematous stroma of loose areolar remnants and cellular debris, with a scattered mononuclear cell infiltration, largely diffused by edema fluid (Fig. 1*A*).

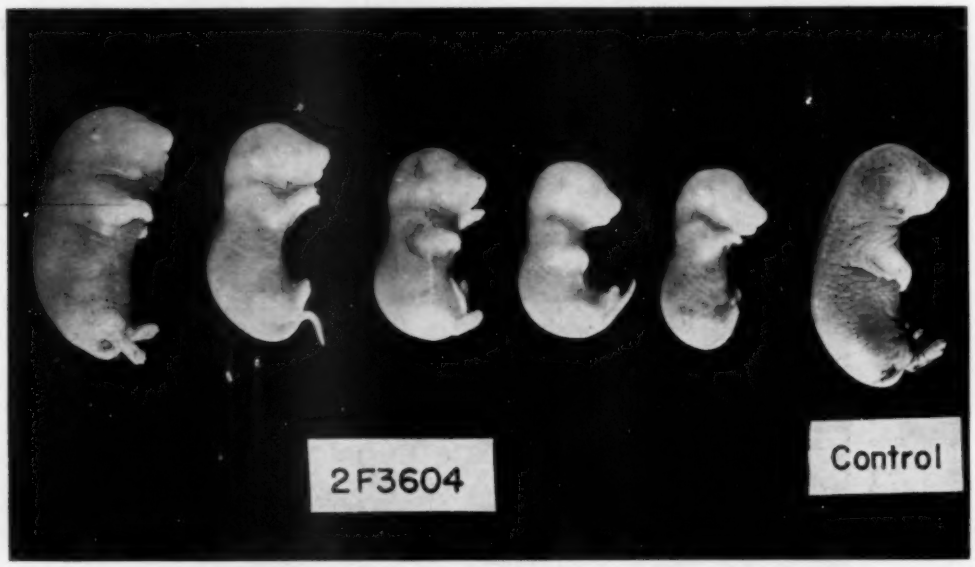


Fig. 2.—A litter of five young (2F 3604) of a rat injected with 650 mg. of ethionine from the 10th to the 22d day of pregnancy. A control animal is seen on the right. Four young are small and underdeveloped, weighing about 2 to 2.5 gm. each. The edematous young on the left weighed 4.3 gm.

RESULTS

The effect of the ethionine injections was evaluated by the mother's reproductive performance, by histologic examination of the maternal pancreas, and by inspection of the young. In general, the results paralleled the amounts of ethionine injected.

GROUP I.—The six females in this group received total doses of ethionine ranging

from 25 to 200 mg. They all had normal pancreases. Five of them produced large litters, netting a total of 54 living young, the average litter size of the controls being 8.4. Two of the fetal pancreases were examined and found to be histologically normal. A sixth rat had a litter consisting of 11 young; of these, 9 were living but small, and 2 were dead and edematous.

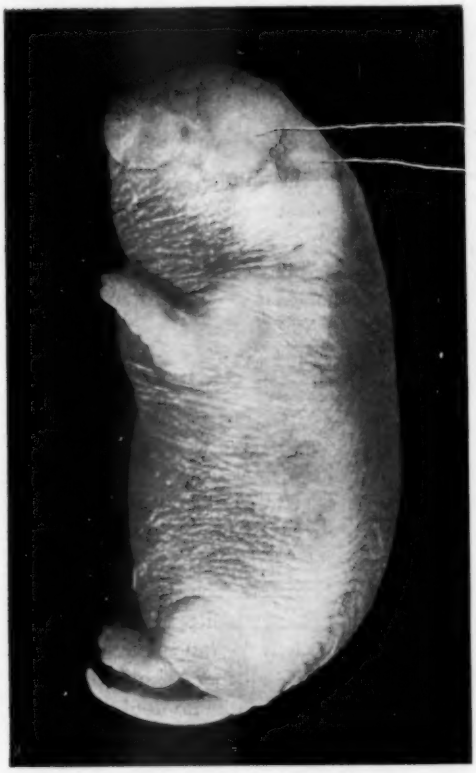


Fig. 3.—Edematous young of ethionine-treated mother.

GROUP II.—Of these 41 females, 6 died during the second half of pregnancy while still receiving injections. Of the remaining 35 mothers, a normal pancreas was found in only 2 of those 30 which were examined. Moderate damage of the pancreas was found in 11 and severe degeneration in 17.

Of the total 35 surviving mothers, 9 resorbed their litters completely. The remaining 26 produced litters totaling 186 young. Only 45 of these young were living and normal in size, whereas 72 were alive but small (Fig. 2). Of the 69 dead young, 10 were of normal size and 59 small. Eight newborn young were markedly edematous (Figs. 2 and 3). Nine young were sectioned serially, and in five of them congenital defects were found. There was a total of three cleft palates, two interventricular septal defects, two ocular anomalies, and one omphalocele in five of the young sectioned. In addition, immaturity of the lungs, the intestine, or the kidney in seven of these young was noted. Extensive hemorrhages were found in the pleural or abdominal cavities of three sectioned young.

GROUP III.—In the 17 rats comprising this group the pancreas was found to be moderately damaged in 2 and severely damaged in 13. The pancreas was not recognizable on dissection in two rats.

These 17 mothers underwent a total of 37 pregnancies, but, because of multiple resorptions, only 6 litters were obtained. None of the 31 young produced were of normal size. Twenty-four were living but small, and 6 were dead and small. One young was dead and very edematous (weighing 4.3 gm., as opposed to an over-all average of 2.0 gm. for the litter).

The pancreases in 26 fetuses derived from 24 mothers belonging to Groups II and III were examined histologically. With the exception of one pancreas, which showed mild signs of pancreatitis, all were found to be normal.

To test whether susceptibility to ethionine differs in different age groups, 22 newborn and 20 weanling rats were subjected to ethionine treatment comparable to that of the pregnant females.

Two litters, comprised of 22 newborn rats, were injected intraperitoneally with 1.0 mg. of ethionine (0.04 cc.) daily from the 1st to the 13th day and killed on the 14th day of postnatal life. Although a 25-gauge needle was used for the injections, there was often a seepage of the fluid admin-

istered, which may explain the variability of the results. Eight newborn rats died before the termination of the injection period. Of the remaining 14, 1 had a normal pancreas and 2 had mild pancreatitis. Four had moderate, and seven, severe degenerative changes, although sometimes areas of relative preservation persisted.

Twenty weanling females, fed an adequate diet, were injected intraperitoneally with 2.5 mg. (0.1 cc.) of ethionine solution daily from the 21st to the 34th day of postnatal life. They were killed on the 35th day. There was one normal pancreas, and mild degeneration was found in two. Moderate pancreatic degeneration was observed in 13 weanlings and moderate to severe degeneration in 4.

COMMENT

These experiments confirm the findings of Father and Popper [4] and of Goldberg and associates,[7] who demonstrated severe damage to the pancreas of rats treated by ethionine injections. Pregnant females receiving 250 mg. of ethionine or more showed, in most instances, pancreatic damage of the type described by other authors. The reproductive performance of these rats was poor. Many of the treated mothers resorbed their young early in pregnancy, and those who carried their litters to or near the end of gestation had many stillborn and undersized young. Such offspring were also retarded in development for their gestational age. Occasionally in a liter of small offspring, one young was of almost normal size and weight. It was found on sectioning, however, that such young were small but enveloped in a coat of edema fluid, which was responsible for the increase in size and for the monstrous appearance (Fig. 2). There was a high incidence of congenital malformations in the nine young subjected to serial sectioning. Yet these malformations were rather varied and did not conform to a definite pattern. The young sectioned cannot be considered representative of the entire offspring of ethionine-treated mothers, since they were selected for sectioning because of their abnormal external appearance. It is not surprising that females with severe ethionine damage show such poor reproductive performance. Their food intake, their digestion, their protein ‡ and lipid metabolism,§ and their plasma and tissue enzymes ‖ are disturbed. Ethionine treatment interferes with the growth of young rats,¶ and it is understandable that it inhibits the growth of rat fetuses. But it is surprising that under experimental conditions which destroy the pancreas of the mother, differentiation and growth of the fetal pancreas are possible. The differentiation of the pancreas begins in the rat embryo on about the 13th day and continues its development and growth throughout fetal life. Ethionine administered to the mother during this time causes severe damage to her pancreatic tissue but does not interfere with the development and growth of the pancreas of her young. We have no explanation for the protection of the fetal pancreas and can only speculate on the protective factors. It is generally assumed that amino acids can pass the placental barrier,[13] but it is possible that ethionine does not reach the embryo in a concentration sufficient to cause damage; or it may be that ethionine is changed by the placental tissue into a nontoxic compound. Finally, it must be remembered that the metabolism of embryonic tissues differs from that of the adult and that an antimetabolite injurious to the maternal tissue may be innocuous to that of the young. While all these explanations are hypothetical, there can be no doubt about the relative resistance of the fetal pancreas to ethionine. In order to demonstrate a possible age factor in this phenomenon of resistance, groups of newborn and weanling rats were treated with intraperitoneal injections of ethionine in doses comparable to those of the mothers on a weight basis. While these young rats had a high mortality, the pancreases of the survivors were generally less injured than those of adult female rats.

‡ References 1, 12, and 14.

§ References 5, 8, 10, and 11.

‖ References 1, 2, and 9.

¶ References 3, 8, and 15.

SUMMARY

Female rats injected with ethionine during pregnancy showed a poor reproductive performance. There were many resorptions and stillbirths, and most of the young found at the end of the gestational period were underdeveloped and markedly reduced in size and weight. The pancreases of the mothers were severely damaged by the ethionine injections, but those of the fetuses remained essentially normal. Neither congenital pancreatic fibrosis nor meconium ileus could be produced in the young by the ethionine injections of the mother.

Dr. Benjamin H. Landing gave help and criticism in the evaluation of the histologic findings.

REFERENCES

1. de Almeida, A. L., and Grossman, M. I.: Experimental Production of Pancreatitis with Ethionine, Gastroenterology **20**:554, 1952.

2. Bollag, W., and Gallico, E.: Effect of DL-Ethionine on the Content of Some Enzymes in Pancreas and Liver, Biochim. et biophys. acta. **9**: 193, 1952.

3. Dyer, H. M.: Evidence of Physiological Specificity of Methionine in Regard to Methylthiol Group: Synthesis of S-Ethylhomocysteine (Ethionine) and Study of Its Availability for Growth, J. Biol. Chem. **124**:519, 1938.

4. Farber, E., and Popper, H.: Production of Acute Pancreatitis with Ethionine and Its Prevention by Methionine, Proc. Soc. Exper. Biol. & Med. **74**:838, 1950.

5. Farber, E.; Simpson, M. V., and Tarver, H.: Studies on Ethionine: Interference with Lipide Metabolism, J. Biol. Chem. **182**:91, 1950.

6. Fitzgerald, P. J., and Alvizouri, M.: Rapid Restitution of the Rat Pancreas Following Acinar Cell Necrosis Subsequent to Ethionine, Nature, London **170**:929, 1952.

7. Goldberg, R. C.; Chaikoff, I. L., and Dodge, A. H.: Destruction of Pancreatic Acinar Tissue by DL-Ethionine, Proc. Soc. Exper. Biol. & Med. **74**:869, 1950.

8. Hardwick, V. L.: Effects of Diethylcholine and Ethionine on Growth Rate, Liver Fat, and Kidney Degeneration of Rats, Proc. Soc. Exper. Biol. & Med. **69**:217, 1948.

9. Henning, N., and Heinkel, K.: Untersuchungen über die Äthioninpankreatitis der Ratte, Ztschr. ges. exper. Med. **120**:221, 1953.

10. Jensen, D.; Chaikoff, I. L., and Tarver, H.: Ethionine-Induced Fatty Liver: Dosage, Prevention and Structural Specificity, J. Biol. Chem. **192**: 395, 1951.

11. Koch-Weser, D., and Popper, H.: Hepatic Fibrosis Produced by Chronic Ethionine Feeding, Proc. Soc. Exper. Biol. & Med. **79**:34, 1952.

12. Levine, M., and Fopeano, J. V., Jr.: Effect of Ethionine on Protein Content of Liver in Growing Rats, J. Biol. Chem. **202**:597, 1953.

13. Needham, J.: Chemical Embryology, New York, The Macmillan Company, 1931, Vol. III.

14. Simpson, M. V.; Farber, E., and Tarver, H.: Studies on Ethionine: Inhibition of Protein Synthesis in Intact Animals, J. Biol. Chem. **182**:81, 1950.

15. Stekol, J. A., and Weiss, K.: Study on Growth Inhibition by D-, L-, and DL-Ethionine in the Rat and Its Alleviation by Sulfur-Containing Amino Acids and Choline, J. Biol. Chem. **179**:1049, 1949.

16. Wachstein, M., and Meisel, E.: Equal Effectiveness of L- and D-Ethionine in Producing Tissue Damage in Rats and Mice, Proc. Soc. Exper. Biol. & Med. **82**:70, 1953.

17. Wachstein, M., and Meisel, E.: Cellular Changes Accompanying Degenerative and Regenerative Phase of Ethionine-Induced Pancreatic Damage in the Rat, Lab. Invest. **2**:253, 1953.

SQUAMOUS METAPLASIA IN THE RAT UTERUS

C. FREDERIC FLUHMANN, M.D., San Francisco

THE OCCURRENCE of so-called squamous metaplasia in the cervix uteri and endometrium of various species, including the human, generally has been considered as resulting from identical pathologic lesions. The present study in the rat indicates that the formation of squamous epithelium in these tissues may be elicited with estrogenic hormone, but two very different histologic processes are involved.

EXPERIMENTAL PROCEDURES

A total of 193 female white rats of the Sprague-Dawley strain and from our own colony were employed in this study.

Eighteen normal rats were killed in order to obtain control specimens of the epithelium of the vagina, cervix, and cornua at ages of 1, 10, and 20 days, during each stage of the estrous cycle, and in early pregnancy.

The experiments were conducted with animals castrated between the ages of 21 and 30 days. Of these, 18 were studied at periods varying from one to three weeks after castration, 76 at intervals of a few hours to 21 days following the single injections of different doses of estrogenic hormone, and 8 after different doses of progesterone. The effects of the injection of diethylstilbestrol 0.5 mg. in oil three times per week were seen in 29 rats treated for from 9 to 195 days. A mixture containing 25.0 mg. of progesterone and 0.5 mg. of diethylstilbestrol per cubic centimeter was administered in 0.1 cc. doses in various ways to 22 animals, of which 6 received this injection three times weekly for as long as 85 days.

From the Department of Obstetrics and Gynecology, Stanford University School of Medicine.

This investigation was supported in part by a research grant (G4034) from the National Institutes of Health, U. S. Public Health Service.

Technical assistance was given by Mr. V. Montesclaros, Mrs. Elsie Kuhl, and Miss Ione Dunn. The photomicrographs are the work of Mr. Paul C. Tracy. The diethylstilbestrol employed was contributed by Eli Lilly & Company and the progesterone (Proluton) by the Schering Corporation.

In addition, 22 rats, also of the Sprague-Dawley strain and hypophysectomized at 30 days of age, were obtained from a commercial laboratory. The completeness of the operation was checked by a three weeks' period of observation of the body weight and gross examination of the skull at autopsy. These rats were employed to repeat certain of the experiments conducted with the castrates—12 after single doses of estrogen, 2 after a mixture of estrogen and progesterone, and 6 after diethylstilbestrol three times weekly, and 2 were uninjected controls.

The hormones were in oil solution and were given by subcutaneous injection. The progesterone used was Proluton (Schering). The estrogen was diethylstilbestrol (Lilly), but estrogenic effects also were obtained with plain sesame oil. With few exceptions each rat was given a subcutaneous injection of colchicine, 0.2 mg., nine to nine and one-half hours before killing, a procedure which holds mitotic figures in the metaphase and greatly enhances the recognition of those formed during the preceding few hours (Dustin's technique). The animals were killed with illuminating gas, and at autopsy the uteri and vaginas were fixed in formalin and stained with eosin-hematoxylin. In each case a section of tissue 1.5 to 2.0 cm. long was taken, which included the upper part of the vagina, both cervices, and the lower ends of the two cornua, and in most instances an additional portion about 1.0 cm. long of each of the two upper cornua also was obtained. They were sectioned horizontally in order to show the epithelial lining. A few sections from the animals which had received chronic treatment with estrogen were stained for glycogen and mucin with the periodic acid-leucofuchsin technique, and for collagen fibers with the Van Gieson stain.

EPITHELIUM OF THE RAT UTERUS

The double uterus of the rat is composed of two distinct types of epithelium: (1) the endometrium, lining each cornu and extending into the corresponding cervix, and (2) the cervicovaginal epithelium, which begins at a point 3 to 5 mm. above each external os and is continuous with the lining of the vagina. There are very definite morphologic and biologic differences between these two mucosae which become apparent under various physiologic conditions or after the administration of the ovarian hormones.

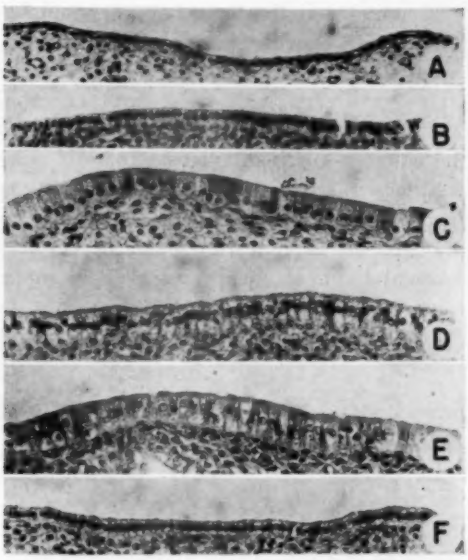


Fig. 1.—Endometrium of the rat under various hormonal influences; reduced ⅔ from mag. × 220.

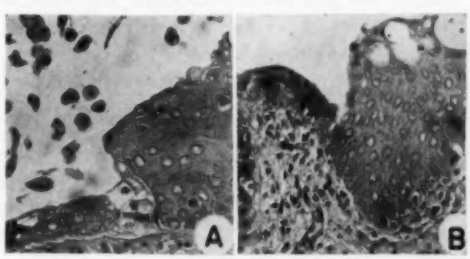
A, three weeks after castration. *B*, immature rat 20 days of age. *C*, 33 hours after single injection of diethylstilbestrol, 0.033 mg. *D*, secretory activity following injection of estrogen and progesterone (48 hours). *E*, estrous phase in normal cycle. *F*, early pregnancy.

The endometrium is of Müllerian origin and is composed of a surface epithelium continuous with tubular glands. It undergoes a number of changes which are valuable criteria of ovarian endocrine function (Allen [1]), but the nature of these transformations is very different from those of the vagina. They involve mostly an increase in size and number of cells, which is induced by estrogens, and a secretory activity controlled by progesterone. These cells are arranged in a single layer, and at birth they are of a small embryonic type. After hypophysectomy or castration they undergo a profound atrophy (Fig. 1*A*), while the glands are collapsed and shrunken, and there is an extensive infiltration of the submucosa with leucocytes. In the immature animal and during diestrus in the adult the cells vary from cuboidal to low columnar and have a dense cytoplasm with a basal nucleus (Fig. 1*B*). Under adequate estrogenic stimulation in the castrate there is an initial period of growth demonstrated by large numbers of mitotic figures in both the glandular and the surface epithelium, and the cells become tall and columnar (Fig. 1*C*). The addition of progesterone results in the appearance of secretory changes in the cytoplasm, and the nucleus is elevated from the base (Fig. 1*D*). During estrus or regression after estrogenic stimulation there is a vacuolation of the cells but, in contrast to regressive processes in the vagina, there is no wholesale desquamation (Fig. 1*E*). The endometrium during pregnancy and pseudopregnancy may correspond to the picture seen after estrogen-progesterone stimulation, but more frequently it is characterized by low columnar cells with secretory activity (Fig. 1*F*).

The vagina is also a Müllerian derivative, but it is now believed that its lining epithelium, and probably that of the lower endocervix, comes from the urogenital sinus. Many changes characterize the various stages of the estrous cycle, pregnancy, and stimulation with ovarian hormones in the immature animal or following hypophysectomy or castration. These histologic transformations are sufficiently marked to alter completely the character of the epithelium, both in morphology and in function. In the newborn infant and after hypophysectomy or castration the cells are small cuboidal elements arranged in one, or usually two, layers. The administration of subminimal doses of estrogen causes a moderate hypertrophy and then a rapid proliferation of the cells until they become arranged in a stratified layer of four to six rows. The surface cells become dif-

Fig. 2.—Junction in the cervix uteri of endometrium (left) and the cervicovaginal epithelium (right) in the rat; reduced ⅔ from mag. × 220.

A, after diethylstilbestrol, 0.5 mg., three times weekly for 37 days. *B*, eight days after a single injection of stilbestrol 0.5 mg.



ferentiated into tall secreting columnar units, the so-called "mucification." During pregnancy or after the administration of progesterone or androgen these mucified cells become stratified and form multiple papillary projections. On the other hand, the administration of one or more units of estrogen causes a disappearance of the mucification and the development of typical squamous epithelium with keratinization. The steps leading to this final transition are described in more detail later in this paper, under the heading "Prosoplasia of the Cervicovaginal Epithelium." Of special significance in comparing this mucosa to the endometrium is the massive desquamation of all the layers, except the lower three or four basal cells, which follows the cessation of ovarian stimulation. This desquamation occurs in both the mucified and the cornified state and is attended with an extensive infiltration with leucocytes and vacuolation and disintegration of the individual cells. It is seen during metestrus in the estrous cycle, following pregnancy and pseudopregnancy, and on cessation of the administration of ovarian hormones.

The endocervix is composed of an upper portion, with endometrial cells, and a lower portion, of vaginal elements. Although it has been maintained that the endocervix contains a "transitional zone" (Migliavacca [2]), a "gradient from the vagina to the uterus" (Hamilton [3]), and, in the mouse, an "intermediate area" (Suntzeff and associates [4]), this difference actually depends only on a varying intensity and time of appearance of the changes induced by the ovarian hormones. With adequate stimulation, the upper part of the cervical canal undergoes variations identical to those of the endometrium, and the lower endocervix responds in the same manner as the contiguous areas of the vagina. It consequently seems more correct to speak of "cervicovaginal," instead of merely "vaginal," epithelium.

The behavior of the two types of epithelium at their junction in the cervical canal demanded special attention because of the possibility that under certain conditions the one might spread and supplant the other. It was found that the line of demarcation between the endometrium and the cervicovaginal epithelium remained clear-cut at all times and even with wide divergences in their endocrine reactions (Fig. 2*A*, *B*). In no case was there an extension of Müllerian epithelium from the cornua down to the portio vaginalis, as has been claimed in the human, or an upward spread of cervicovaginal tissue in the presence of an intact endometrium. However, it does appear that squamous epithelium from the cervix can extend into the cornua, but only after the extensive destruction of the endometrium which follows chronic treatment with estrogenic hormones.

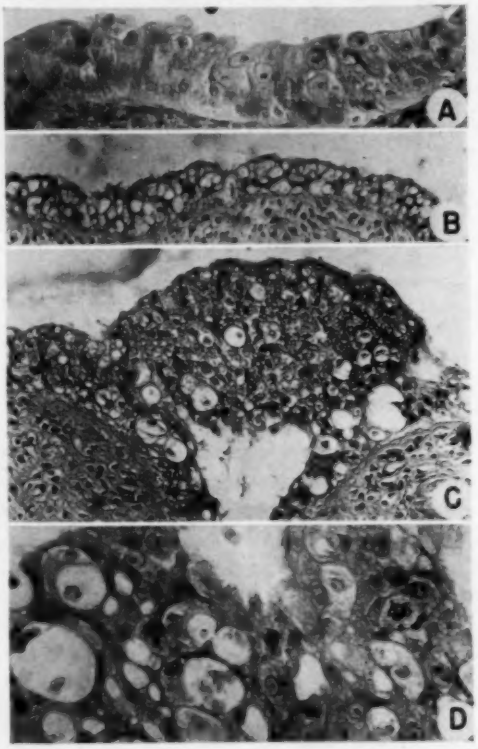


Fig. 3.—Endometrium of rats receiving one injection of diethylstilbestrol, 0.5 mg., three times per week.

A, 96 hours after first injection; reduced 2/5 from mag. × 220. *B*, vacuolation of cells (16 days); reduced 2/5 from mag. × 220. *C*, degeneration with polypoid outgrowth (37 days); reduced 2/5 from mag. × 220. *D*, mitosis in area of degeneration (37 days); reduced 2/5 from mag. × 440.

SQUAMOUS METAPLASIA OF THE ENDOMETRIUM

The induction of squamous metaplasia in the cornua of the rat uterus was first accomplished with estrogenic hormone by Selye, Thomson, and Collip [5] and has been reproduced by several subsequent investigators.* In the present study, the rats developing this change received one subcutaneous injection of diethylstilbestrol 0.5 mg. in oil, three times a week. This is a large dose, for a single injection causes the vagina to become cornified in 36 to 40 hours and remain in this stage for one week, and reparative processes are not completed until the 12th day.

The changes preceding the appearance of squamous epithelium are quite remarkable and give a clue to the manner in which this transition takes place. The first effect results from the growth-stimulating activity of estrogenic hormone and is well advanced within the first 24 hours. The atrophied cells of the castrate rat uterus rapidly hypertrophy, mitotic figures appear in large numbers, and there is an increase in the number of cells (Fig. 3*A*). They become very tall and broad, with some distended with clear fluid and the nucleus compressed and forced aside, so that they bulge between neighboring cells. Korenchevsky and Hall [12] have described them as "tumbler cells." In order to accommodate this active growth, the cells become stratified into two to six layers and form small tufts projecting above the surface. The folds of the endometrium do not attain the degree reached during pregnancy, but they do persist, although less prominently, during the subsequent weeks and finally disappear after some four months.

The first striking characteristic of this process is thus a growth activity, but within a few days evidence of the degeneration described in this paper makes its appearance. This combination of active growth with disintegration leads to many bizarre histologic pictures.

After one week of intense proliferation large numbers of cells show vacuolation. This change was clearly present on the ninth day, and one week later these changes had become intensified and widespread (Fig. 3*B*). They resemble those occurring in the regression which follows single hormone injections. The degeneration is further manifested by many cells with swollen, coarsely granular cytoplasm and indistinct outlines (Fig. 3*C*).

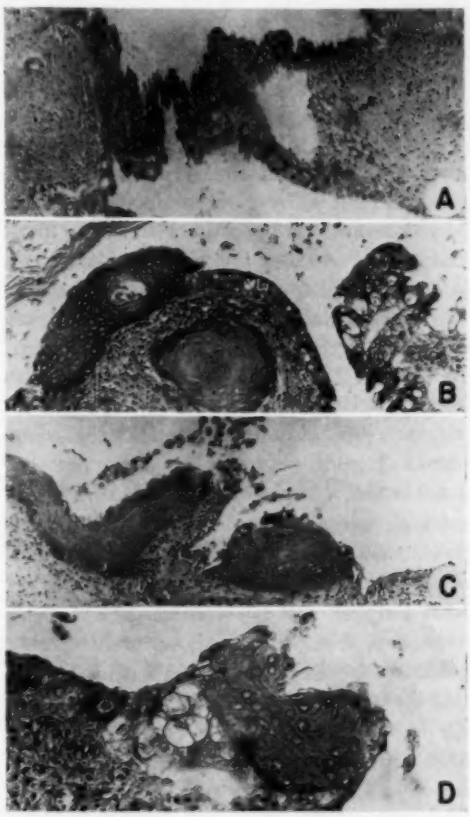


Fig. 4.—Endometrium of rats receiving one injection of diethylstilbestrol, 0.5 mg., three times per week.

A, area of degeneration forming polypoid outgrowths (120 days); reduced ⅔ from mag. × 112. *B*, squamous metaplasia on surface and in gland (37 Days); reduced ⅔ from mag. × 112. *C*, squamous metaplasia on surface epithelium (108 days); reduced ⅔ from mag. × 112. *D*, squamous metaplasia in area of degeneration (30 days); reduced ⅔ from mag. × 220.

Some nuclei appear normal, but many show karyorrhexis and pyknosis. Normal cells are scattered throughout, either singly or in small clumps, and a strange finding in such a picture is that of mitotic activity (Fig. 3*D*).

* References 2, 6-11, and 13.

This combination of growth and degeneration at first is seen here and there along the surface, and stratified projections jut into the lumen, forming polypoid outgrowths (Fig. 4*A*). In time the whole uterine cavity and the upper endocervix are involved. Smaller and larger areas become separated from the underlying submucosa. Leucocytes and epithelial cells, singly or in clumps, are desquamated free into the lumen. The submucosa becomes hyalinized; this change is advanced in 30 days and is accompanied by a diminution in the number of glands.

These areas of degenerating tissues with intermingled normal cells and cells with mitotic figures have been interpreted by Migliavacca [14] as "syncytium-like" structures resulting from a combined stimulation by estrogen and anterior pituitary gonadotropin. He described them as occurring in the rat during prolonged treatment with estrogen, and Novak [15] has found similar lesions in the human accompanying hyperplasia of the endometrium. My reasons for regarding them as primarily the result of a degenerative process are (1) that they are found in a less marked form during the stages of estrus and metestrus of the normal cycle; (2) that they occur during regression after stimulation with a single dose of estrogen; (3) that the individual constituents show unmistakable evidence of cellular disintegration in nucleus and cytoplasm, and (4) that the vacuoles in the individual cells do not contain glycogen or mucin, which would indicate functional activity. They also are not solely an estrogenic effect because a similar break-down is seen after long-continued treatment with androgen, and anterior pituitary gonadotropin is not essential, since these changes occur in hypophysectomized animals given estrogen.

The first evidence of squamous epithelium is found in small foci either on the surface or deep in the glands (Fig. 4*B*). The foci are composed of small clumps of hexagonal cells with clear outlines, distinct nuclei, and well-stained cytoplasm. Sometimes they assume a concentric arrangement. In most cases they lie directly on the submucosa (Fig. 4*C*), and sometimes they are in the center of degenerating areas (Fig. 4*D*). The squamous epithelium from these foci grows out along the exposed surface, once it is firmly established on the submucoa, but some can be seen desquamated into the lumen along with other cellular detritus. The squamous epithelium of the cervicovaginal portion also may extend upward into the cornua, but only after the surface endometrium has been destroyed. This extension occurs as a migration of the whole squamous epithelium, and no evidence was seen of extension by a subepithelial infiltrative growth of the basal layer.

Islands of squamous epithelium were found in varying numbers in all the animals killed after nine days. A complete transformation of both cornua was not obtained in this series, but long strips of metaplastic epithelium were seen after 103, 179, 188, and 195 days. The cellular degeneration with desquamation into the lumen, rather than the metaplastic process, always dominated the picture. Two rats, one at 188 and one at 195 days, at autopsy showed very large swollen cornua, which suggested pyometra. The contents, however, were composed chiefly of milky-white secretion with lamellae of keratinized cells.

The six hypophysectomized rats treated in this manner with estrogen were killed at 21, 32, 47, and 60 days, and two died at 30 and 31 days, respectively. The histologic changes did not differ from those found in the castrates, but the destruction of the epithelium was very profound and squamous metaplasia occurred only in small islands. At 21 days the cornua were much distended with white material containing keratinized lamellae. In the two animals which died and the two killed at 47 and 60 days, enormous pyometra were found, with almost complete destruction of the lining epithelium.

Since only six rats were treated for long periods of time with a mixture of diethylstilbestrol and progesterone, definite conclusions cannot be made regarding the findings. However, progesterone is believed to inhibit the formation of squamous epithelium; hence

it is important to note that extensive squamous metaplasia was seen on the surface and in the glands in three animals killed after 30, 46, and 85 days, respectively. A degeneration of the lining epithelium was observed in all the rats treated in this manner, but to a less degree than in the estrogen series, and the proliferative changes were more in evidence. The endometrium was thrown up into large folds, the glands were hyperplastic and many were cystic, and the individual cells showed marked hypertrophy and stratification.

Since there is apparently no information available regarding healing processes in the endometrium after prolonged treatment with estrogen, eight rats were set aside for this purpose. They were given one injection of 0.5 mg. of diethylstilbestrol three times a week for 164 days, and one rat was killed at 9, 16, 25, 39, 51, 64, 78, and 94 days, respectively, following the cessation of treatment. Since the estrogen was in an oily solution, a stimulating effect continued for at least 25 days, judging by the profusion of mitotic figures in the basal cells of the vaginal squamous epithelium. These cells undergoing mitosis finally disappeared after the 39th day.

The induction of islands of squamous epithelium in the endometrium by prolonged estrogenic hormone stimulation is a reversible reaction. The foci of squamous metaplasia disappeared promptly after discontinuation of the injections. They were not seen in any of the eight animals, a rather striking finding in view of their constant presence after the ninth day during the period of treatment. The degenerative processes in the epithelium with massive desquamation of the cells continued until the 39th day, and there was a pronounced infiltration of the submucosa with leucocytes, eosinophiles, and macrophages laden with blood pigment. This picture gradually diminished in intensity, but cells with vacuolation and leucocytic infiltration were still apparent at 94 days. The appearance of large numbers of cells considered normal for the castrate could be seen after 39 days, and coincidently there were numerous mucosal folds and a progressive development of glands. A resorption of hyaline tissue with development of a normal submucosa was seen after the 25th day, but even at 94 days this feature of the reparative process could not be considered as complete.

PROSOPLASIA OF CERVICOVAGINAL EPITHELIUM

The cyclic formation of squamous epithelium in the vagina of various species during the estrous cycle has been recognized since the pioneer studies of Stockard and Papanicolaou in the guinea pig, Long and Evans in the rat, and Edgar Allen in the mouse. This process is not considered as "squamous metaplasia" in the rodent, but the term has been applied to some lesions of the cervix and the prostate, in the human and in other species, which seem to develop in the same manner and from the same endocrine stimulus (Fluhmann [16]). Consequently, it is desirable to apply a designation distinct from "metaplasia" to this change. The term "prosoplasia," derived from two Greek words meaning "forward" and "to form," seems appropriate and was used in a previous communication [16] as referring to the orderly evolution of the cervicovaginal epithelium under endocrine stimulation. This definition differs from its original meaning, but not in a contradictory sense. It apparently was first employed in botany by Küster as referring to a highly specialized differentiation of some plant growths. Schridde in 1907 then used it as connoting the development of certain cells beyond the usual normal limits, for instance, excessive keratinization of the skin in pachydermia and leucoplakia (Fischer-Wasels [17]).

The various "prosoplastic" changes in the rat or mouse cervicovaginal epithelium which lead to the formation of squamous epithelium have been employed as a test for estrogenic hormone. The usual procedure is to study the cytology of vaginal scrapings, but in the "Fluhmann mucification test" microscopic sections of the vagina are employed.[16] The various steps leading to this end-point have been classified into as many as seven categories. However, these responses in the

hypophysectomized or castrated rat can be simplified into four distinct stages.

Stage 1.—Small atrophic or immature undifferentiated cells in one or two layers. They are seen in very young, castrated, and hypophysectomized rats or mice. After the administration of estrogen they first hypertrophy and then begin to proliferate (Fig. 5*A*).

Stage 2.—The epithelium is composed of a surface layer of columnar mucous cells of low or medium height, with an underlying stratum of small cuboidal cells. At first composed of only one or two rows of cells, this basal layer increases in depth until the columnar cells at the surface are undermined by several rows of cuboidal elements (Fig. 5*B*).

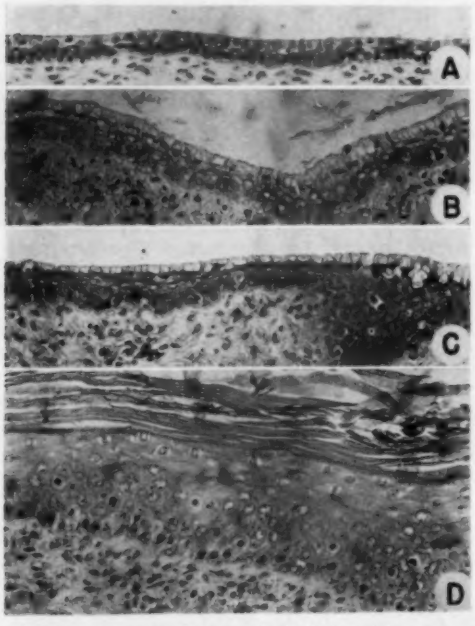


Fig. 5.—Stages of prosoplasia induced by estrogen in the cervicovaginal epithelium of castrate rat; reduced ⅔ from mag. × 220.

A, Stage 1, early hypertrophy of surface cells. *B*, Stage 2, surface layer of columnar cells with underlying small basal cells. *C*, Stage 3, early differentiation of basal cells into squamous epithelium. In this case the surface columnar cells have not yet been desquamated. *D*, Stage 4, squamous epithelium with keratinization.

Stage 3.—The stratified cuboidal cells now undergo differentiation into squamous epithelium. The surface rows become elongated, with the long axis parallel to the surface, and keratinization begins. A middle layer of deeply stained hexagonal cells develops. The basal cells remain small but their long axis becomes directed vertically. The surface mucous columnar cells are desquamated at some time during this process but they may persist while differentiation takes place in the layers beneath them (Fig. 5*C*).

Stage 4.—The final stage presents the normal three layers of well-differentiated squamous epithelium with keratinization (Fig. 5*D*).

A complete Stage 4 of squamous epithelium is accomplished in 36 hours after the administration of one or more units of estrogen. The general pattern does not change with prolonged treatment, even after 195 days, except for the persistence of many mitotic figures in the basal layer and excessive desquamation of cornified cells. After discontinuation of the hormone there is an extensive vacuolation of the cells of the two outer layers of the squamous epithelium and infiltration with leucocytes. In the castrates treated for 164 days, this disintegrating process could be seen as long as 94 days after the injections had been discontinued.

COMMENT

The evidence advanced in these experiments appears to justify the concept that "squamous metaplasia" in the uterus of the rat is accomplished by two very distinct processes according to the epithelium involved.

The prolonged administration of estrogenic hormone results in an extensive degenerative lesion of the endometrium with the appearance of cells, which may correctly be considered as "squamous metaplasia." It is, possibly, in the nature of a reparative process and is not a specific estrogenic effect, since it also can result in the castrate from treatment with estrogen and progesterone, with androgen (Korenchevsky and Hall [12]), and in the intact animal with chorionic hormone (Fluhmann [19]). Wolbach and Howe [20] found identical lesions in the rat in vitamin

A deficiency, and in other animals it has been produced in many organs and tissues as a result of atrophy, chronic irritation, and inflammation (Fluhmann [16]).

On the other hand, the induction of squamous epithelium in the cervicovaginal mucosa is an orderly, progressive cellular development brought about only by estrogenic hormone. The term "prosoplasia" is suggested for this process.

The usual explanation for the regeneration and metaplastic transformation of tissues such as the epithelium of the rat uterus, is that it is brought about by multipotent cells which exist as embryonic inclusions. This concept seemingly evolved from botanical studies which drew attention to "cambium cells" arising from undifferentiated zones and held responsible for the growth of the tissues. The present studies of "squamous metaplasia" and "prosoplasia" in the rat endometrium and cervicovaginal epithelium do not disprove the possible existence of "indifferent cells" leading to such changes. Nevertheless, it does seem an unnecessary theory, and a simpler explanation is that the "indifferent cells" actually are the normal cells of the epithelium at a stage in their development when they are sufficiently mature to divide by mitosis but before their complete differentiation, when they are unable to multiply. In other words, at one time in its life cycle each normal cell, whether it eventually becomes columnar or squamous or simply undergoes multiplication, is an indifferent cell. This observation applies particularly to prosoplasia of the cervicovaginal mucosa, where squamous epithelium appears cyclically, but also may be applicable to the endometrium. The tremendous numbers of mitotic figures elicited by a single dose of estrogen suggests that the normal cells themselves are endowed with a multipotency which enables them to respond in one direction or the other as a result of proper stimulation. For example, Allen, Smith, and Gardner,[21] using the colchicine technique, saw as many as 1,585 mitotic figures in one transverse section of the immature mouse vagina 37 hours after a single injection of estrogen. The appearance of such sections makes one doubt very much that special units for proliferation must be present, since there are almost as many cells with mitotic figures as normal cells.

The differentiation into two distinct processes leading to so-called "squamous metaplasia" is not merely of academic interest, since these findings in the rat may be used as prototypes for similar lesions in many organs and tissues of other species, including the human (Fluhmann [16]). The development of islands of squamous metaplasia, such as described here, is found widely distributed (lungs, bronchi, esophagus, gall bladder, endometrium) and follows chronic inflammation, adenocarcinoma, atrophy, mechanical irritation, trauma, excessive and prolonged hormonal stimulation, and vitamin A deficiency.

In contrast to metaplasia, various stages of a process comparable to prosoplasia in the rat have been found both in the human and in other experimental animals as a result of estrogenic hormone stimulation. They have been observed in the urethra, paraurethral glands, Bartholin's glands, vagina, and cervix uteri of the female of many species. In the male they have been found, and induced experimentally with estrogens, in the urethra, prostate, Cowper's glands, and seminal vesicles of men and many animals. There is also evidence that in both sexes this distribution is limited to organs and tissues which are derived from the embryonic urogenital sinus (Zuckerman [22]; Fluhmann [16]).

SUMMARY

A conversion of the mucosa of the rat uterus into squamous epithelium is brought about with estrogenic hormone by one of two different processes according to the area involved.

1. A squamous metaplasia of the endometrium occurs as an indirect effect following prolonged treatment with large doses of estrogen. There is, first, an extensive degeneration of the epithelium, and multiple small islands of squamous cells appear which eventually may fuse together and partly line the uterine cavity.

2. The cervicovaginal epithelium may be converted in a short time into squamous epithelium by relatively small doses of estrogen. It is a progressive orderly transformation of the whole mucosa, and is a direct hormonal effect. The term "prosoplasia" is suggested for this process.

These changes are probably induced because of multipotent properties of the original cells and do not necessarily require the presence of primitive so-called "indifferent" cells.

REFERENCES

1. Allen, E., in Sex and Internal Secretions, Ed. 2, Baltimore, Williams & Wilkins Company, 1939.

2. Migliavacca, A.: Inkretogene heterotype Epithelwucherungen im Uterus, Arch. Gynäk. **162**: 595, 1936.

3. Hamilton, C. E.: Cervix Uteri of the Rat, Anat. Rec. **97**:47, 1947.

4. Suntzeff, V.; Burns, E. L.; Moskop, M., and Loeb, L.: On the Proliferative Changes Taking Place in the Epithelium of the Vagina and Cervix of Mice with Advancing Age and Under the Influence of Experimentally Administered Estrogenic Hormones, Cancer **32**:256, 1938.

5. Selye, H.; Thomson, D. L., and Collip, J. B.: Metaplasia of Uterine Epithelium Produced by Chronic Estrin Administration, Nature **135**:65, 1935.

6. Lacassagne, A.: Modifications progressives de l'utérus de la souris sous l'action prolongée de l'oestrone, Compt. rend. Soc. de biol. **120**:1156, 1935.

7. McEuen, C. S.: Metaplasia of Uterine Epithelium, Produced in Rats by Prolonged Administration of Oestrin, Cancer **27**:91, 1936.

8. McEuen, C. S.; Selye, H., and Collip, J. B.: Some Effects of Prolonged Administration of Oestrin in Rats, Lancet **1**:775, 1936.

9. Grumbrecht, P.: Schleimhautveränderungen (Metaplasie) des Uterus bei Dauerzufuhr von Follikelhormon, Arch. Gynäk. **160**:525, 1936.

10. Kaufmann, C., and Steinkamm, E.: Über die Wirkung fortgesetzter Zufuhr unphysiologischer Mengen Follikelhormon auf das Genitale weiblicher Ratten, Arch. Gynäk. **162**:553, 1936.

11. Zondek, B.: Effect of Long-Continued Large Doses of Follicle Hormone upon the Uterus of the Rat, Am. J. Obst. & Gynec. **33**:979, 1937.

12. Korenchevsky, V., and Hall. K.: Bisexual and Co-operative Properties of the Sex Hormones, J. Path. & Bact. **45**:681, 1937.

13. Emge, L. A.: Estrogenic Hormones and Carcinogenesis, Surg., Gynec. & Obst. **68**:472, 1939.

14. Migliavacca, A.: Über die Umwandlung des Uterusepithels in syncytiale Struktur unter hormonalen Einflüssen, Arch Gynäk. **159**:172, 1935.

15. Novak, E.: Gynecologic and Obstetric Pathology, Ed. 3, Philadelphia, W. B. Saunders Company, 1952.

16. Fluhmann, C. F.: Comparative Studies of Squamous Metaplasia of the Cervix Uteri and Endometrium, Am. J. Obst. & Gynec., **68**:1447, 1954.

17. Fischer-Wasels, B.: Metaplasie und Gewebsmissbildung, in Handbuch der normalen und pathologischen Physiologie, edited by A. Bethe and others, Berlin, Springer-Verlag, Vol. 14, Pt. 2, 1927.

18. Fluhmann, C. F.: New Procedure for the Demonstration of Estrin in the Blood of Women, Endocrinology **18**:705, 1934.

19. Fluhmann, C. F.: Comparative Studies of Gonadotropic Hormones: VI. Some Effects of Long-Continued Daily Injections, Endocrinology **25**:193, 1939.

20. Wolbach, S. B., and Howe, P. R.: Tissue Changes Following Deprivation of Fat-Soluble A Vitamin, J. Exper. Med. **42**:753, 1925.

21. Allen, E.; Smith, G. M., and Gardner, W. U.: Accentuation of the Growth Effect of Theelin on Genital Tissues of the Ovariectomized Mouse by Arrest of Mitosis with Colchicine, Am. J. Anat. **61**:321, 1937.

22. Zuckerman, S.: Embryological Interpretation of Changes Induced by Oestrogens in Male Reproductive Tract, Lancet **1**:135, 1936.

EFFECT OF SWEET PEA MEAL ON THE RAT AORTA

THOMAS E. BACHHUBER, B.S.
and
JOSEPH J. LALICH, M.D., Madison, Wis.

SPORADIC outbreaks of lathyrism were first observed in man * and later in cattle.[3] Lathyrism in man is characterized by muscle weakness, paresthesia, and transient paralysis of the lower extremities.[2] The disease has also been produced in rats by feeding various species of pea meal.† In rats the toxic Lathyrus factor produces systemic osteoporosis, moderate to severe skeletal deformities, and hindlimb muscle spasticity with or without paralysis. In addition to skeletal deformities, Ponseti and Baird observed dissecting aortic aneurysms in rats fed sweet pea (Lathyrus odoratus) meal.[9] The arterial changes produced by feeding are significant because over 50% of the rats died of aortic rupture. Any factor present in food which is capable of exerting such a profound influence on the development of bone and the aorta is of general biologic interest. Utilization of the Lathyrus diet therefore offers an unusual opportunity to study the character of the arterial changes. The present study was undertaken to determine the initial site of injury of the aorta and to analyze the factors concerned in the production of the arterial alteration.

METHOD

Sprague-Dawley rats of both sexes, weighing between 36 and 62 gm., were used. Ground pea meal twice extracted with water and alcohol was fed to 17 control rats. Ground crude pea meal ‡ at a similar concentration was fed to 32 test rats.

Test Diet

Casein, plain (Borden's)	10%
Brewers' yeast (Pabst)	10%
Crude pea meal	50%
Cerelose	24%
Salt, Wesson	4%
Olive oil	2%

The following were added to the olive oil per kilogram of diet: 0.21 mg. of vitamin A acetate, 0.26 I. U. of vitamin D, 10 mg. of α-tocopherol, and 0.15 mg. of menadione (2-methyl-1,4-naphthoquinone). The control diet was similar to the test diet except for the substitution of water-alcohol-extracted pea meal for the crude meal. Both groups were fed ad libitum. A few control animals of similar age were killed and examined to compare with test rats. Each animal was inspected for deformities of the sternum, vertebral column, and femur. The aortae were examined for aneurysmal dilatation or rupture. The tissues were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, Weigert's method for elastic tissue and Mallory's stain for connective tissue.

RESULTS

The weight gains in the two groups and the cause of death in 20 of the 49 rats are shown in the accompanying Table. The control and test rats included in the Table were chosen to illustrate the differences in response to the two diets which were fed.

Gross Observations.—Neither bony deformities nor aortic rupture was observed in 17 control rats, 2 of which died after diarrhea and malnutrition. As illustrated in the Table, extracted pea meal promotes better weight gains than the crude meal. All test rats developed moderate to severe skeletal deformities. Partial hindlimb paralysis secondary to vertebral column deformity occurred in six rats. Of the 28 test rats, aortic aneurysm or rupture was observed in 20 instances. Fifteen rats died of aortic rupture

Preliminary report submitted to *Science*, June 9, 1954.

Supported in part by the Wisconsin Alumni Research Foundation.

Department of Pathology, University of Wisconsin Medical School.

* References 1 and 2.

† References 4 through 8.

‡ Obtained from Ferry-Morse Seed Company, Detroit.

with massive hemothorax. Six died after diarrhea and malnutrition. Three of these had aortic aneurysms, and two had abdominal hernias. In three other rats which apparently died of malnutrition, two had bleeding into the urinary bladder and one had a fractured leg. Four rats were killed, two of which had aortic aneurysms. Four rats were fed commercial pellets and are recuperating from malnutrition. At autopsy, only minimal

Effect of Crude Pea Meal on the Aorta

No.	Days on Diet	Weight, Gm. Initial	Weight, Gm. Final	Cause of Death	Microscopic Examination of Arch: Medial Edema	Fragmented Elastic Fibers	Medial Hemorrhage	Intimal Fibrosis	Mural Thrombus
				Control					
126	41	41.9	135.0	Killed	—	—	—	—	—
127	37	41.8	134.0	Killed	—	—	—	—	—
128	101	59.1	162.0	Killed	—	—	—	—	—
129	101	54.1	184.0	Killed	—	—	—	—	—
147	49	48.6	.....	Died	+	+	+	+	—
148	32	50.4	128.0	Killed	+	+	+	+	—
149	61	42.6	143.3	Killed	+	+	—	+	—
150	28	48.3	125.1	Killed	—	—	—	—	—
165	58	44.0	114.3	Killed	—	—	—	—	—
166	59	43.0	103.3	Killed	—	—	—	—	—
				Test					
122	36	40.8	105.0	Ruptured aorta	+	+	—	+	—
124	38	39.9	119.0	Ruptured aorta	+	+	+	+	—
125	61	36.0	68.0	Malnutrition	+	—	—	—	—
136	43	59.4	148.0	Ruptured aorta	+	+	+	—	—
137	43	61.7	.....	Ruptured aorta	+	+	+	+	—
140	27	59.0	125.0	Ruptured aorta	+	+	+	—	—
141	48	47.1	95.0	Diarrhea aneurysm	+	+	+	—	+
142	52	50.0	87.7	Fractured femur	+	—	—	—	—
160	35	46.2	104.7	Killed	—	—	—	—	—
163	57	42.7	68.2	Diarrhea hematuria	+	—	—	—	—

subcutaneous and retroperitoneal fat was observed in the test rats. Bronchopneumonia was observed in only 2 of 49 animals. All but one of the aneurysms originated in the ascending or the transverse aorta. Neither aneurysmal dilatation nor intravascular hemorrhage was observed in the abdominal aorta.

Microscopic Findings.—Since all the lesions were observed in the region of the arch, it seems desirable to describe this area in some detail. In the ascending, descending, and abdominal aortae the prominent undulating elastic fibrils are parallel to the axis of the aorta. On the other hand, in the arch the elastic fibers are irregularly aligned with respect to the lumen. This peculiar alignment is undoubtedly related to the origin of large vessels at this site. The spaces between the elastic fibers contain a hyaline material composed of collagen fibers, ground substance, and smooth muscle. The intima and adventitia are poorly developed structures in a rat aorta, and so our description refers largely to media. Variations of the aorta at its origin were noted. The aorta may be supported by cardiac muscle, connective tissue, or fat in varying degrees (Figs. 3 and 4). At times medial width was not reduced, whereas in almost half of the rats moderate reductions in caliber were observed (Fig. 4).

The early alterations which were observed in rat aortae probably occurred in the fol-

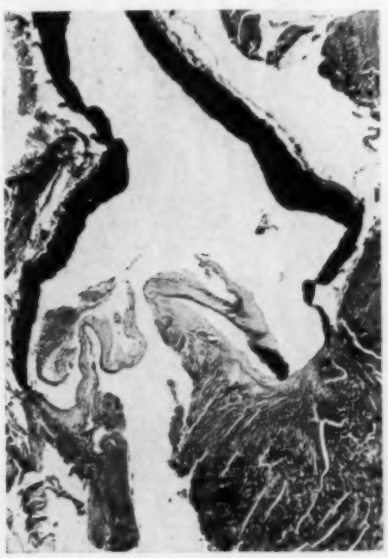
Fig. 1.—Rat 158 died of aortic rupture and hemothorax after a diet of crude pea meal for 35 days. Note the massive hemorrhage around the arch and thoracic aorta.

lowing sequence. Medial edema and swelling followed by fragmentation of the elastic fibers appear first. Once the media is sufficiently weakened, dilatation or dissecting medial hemorrhage may develop. After aortic dilatation, the focus of weakness may be repaired by intimal, medial, or adventitial granulation tissue leading to fibrosis. It was noted that when the dissecting medial hemorrhage was rapidly progressing healing by fibrosis was absent or minimal. Even though medial hemorrhage and aneurysmal dilatation were often associated, either process could occur independently. Inflammatory cells were present only when medial rupture

Fig. 2.—Rat 162 died after aortic rupture after 46 days on the test diet. Heart, lungs, and thoracic blood clot. The rupture occurred in an aneurysm of the aortic arch. The clotted blood found in the thorax has formed a cast of the heart.

Fig. 3.—Rat 125 died of malnutrition after 61 days on crude pea meal. Section taken through aorta, aortic valve, and base of the left ventricle. The aorta is normal in this area. The photograph shows variation in the thickness of the media and the attachment of the myocardium on the adventitial surface. Weigert elastic tissue stain; × 60.

Fig. 4.—Rat 167 killed after 67 days on control diet. Section includes the aorta, the valves, and the base of the heart. The support of the aorta by myocardium and the width of the media are variable. Such variations are normal. Weigert; × 35.



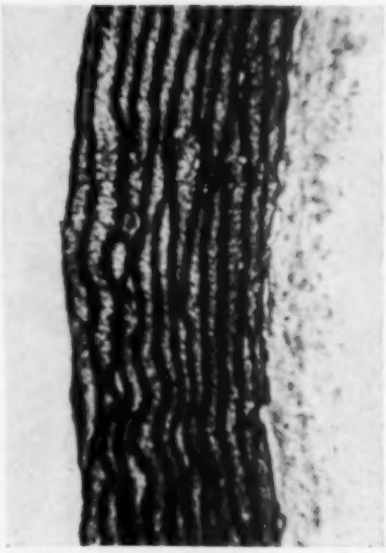


Fig. 5.—Section of thoracic aorta from the same animal. Note that the adventitia on the right and the intima are poorly developed structures in a rat aorta. The interstices are relatively inconspicuous, whereas the elastic fibers constitute the major component of the media and delineate its borders. Weigert; × 300.

Fig. 6.—Aorta taken from thoracic region of Rat 125 (Fig. 3). The increased width of the media is due to enlargement of the interstices by edema fluid. Weigert; × 300.

and periaortic hemorrhage were contained by adventitial fibrosis. The incidence of significant alterations of the aorta is as follows:

	17 Control	28 Test
Edema	4	12
Fragmentation of elastic fibers	4	15
Medial hemorrhage	2	13
Intimal fibrosis	2	10
Hyaline thrombi	0	3

COMMENT

We believe that the Lathyrus diet exerts a marked influence on the integrity of the rat

Fig. 7.—Rat 127 killed after 37 days on the control diet. Section of the transverse arch and a large branch of a normal aorta. At the origin of a major arterial branch the elastic fibers are irregularly aligned with respect to the aortic lumen. Weigert; × 70.

aorta for several reasons. In previous studies even minor alterations of the aorta in senile rats were rarely encountered.[10] Spontaneous sclerotic alterations have been observed in pulmonary, coronary, and mesenteric arteries.§ In our study the majority of 3- to 4-week-old rats developed marked degeneration and medial hemorrhage of the aorta within 27 to 68 days, when fed crude pea meal.

§ References 10 and 11.

The toxic factor in sweet peas was first isolated by Dupuy and Lee [12] and later identified and synthesized by Schilling and Strong.[13] The toxic Lathyrus factor is β(N-γ-L-glutamyl) aminopropionitrile. The synthetic compound prepared by these workers was found to be equally as effective in producing osteoporosis and skeletal deformities as the crystalline extract or crude pea meal. ‖ At present it is not certain whether the toxic factor alone is capable of producing arterial degeneration or whether it may be accelerated by the other constituents which are included in the diet. There are several observations to suggest that the effect of the toxic Lathyrus factor on arteries is modified by both the type and the concentration of proteins included in the diet. For example, in a previous study, when crude pea meal or extracts of pea meal were fed to rats, aneurysms were observed in 3 of 60 test rats.[14] Examination of the diets used disclosed the fact that only rats which were fed crude pea

Fig. 8.—Rat 136 died after hemothorax after 43 days on the test diet. Section of aorta and aortic valve. There is separation of the elastic fibers by a dissecting hemorrhage in the ascending aorta. The valve does not contain elastic fibers. Weigert; × 125.

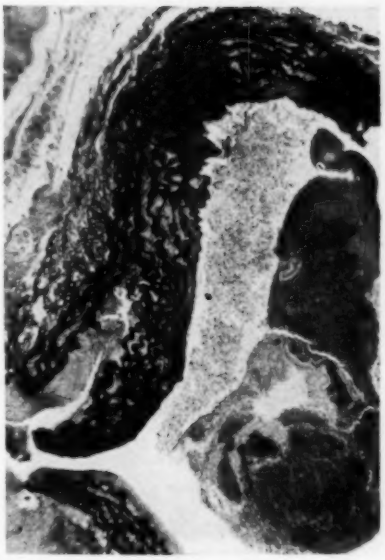
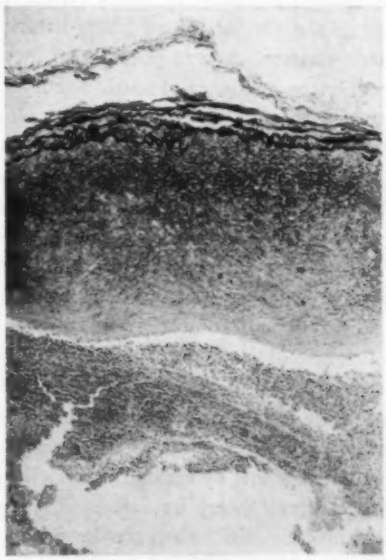


Fig. 9.—Rat 143 died of aortic rupture after 31 days on crude pea meal. Section taken through the arch. At the site of rupture the elastic fibers are fractured. Adventitial fibrosis in this case is absent. Weigert; × 200.

Fig. 10.—Rat 149 killed after 61 days on the control diet. Section through the aortic arch. The elastic fibers in the media are fragmented, and marked intimal fibrous hyperplasia has occurred. Weigert; × 150.



‖ Schilling, E. D.; McKay, G. F., and Strong, F. M.: Unpublished observations.

Fig. 11.—Rat 144 died of aortic rupture after 31 days on the test diet. Section through the ascending arch. In this case there are both intimal and adventitial fibrosis in response to injury. Hematoxylin and eosin stain; × 150.

meal or extracts in combination with 10% casein developed aneurysms. When Ponseti and Baird produced aneurysms in rats, they fed either brewers' yeast and pea meal, alone or in combination with 10% casein.[9] These authors did not consider the possibility that the associated crude casein might be exerting an inhibitory effect on the toxic factor in the production of either arterial or osseous change. It was possible to increase markedly the incidence of aortic aneurysm or rupture in the present study by employing 10% casein. This finding is in keeping with Dasler's observation that casein exerts some protective effect against the development of osteoporosis in Lathyrus-fed rats.[8] Medial hemorrhage in two control rats may have been due either to incomplete extraction of the water-alcohol soluble toxic factor or to a deficiency of some other essential dietary constitutent. We favor the latter possibility because the bones, on gross inspection, were normal in the control rats. Irrespective of the mechanisms by which the toxic Lathyrus factor may exert its influence, there is no doubt that this substance in crude pea meal accelerates and exaggerates the development of aortic degeneration.

Comparison of changes observed in rat aortae fed the Lathyrus factor with human arteriosclerosis indicates that some similarities are present. Aneurysmal dilatation with or without intimal hyperplasia, which was observed in rats, is also seen in human arteriosclerosis.[10] The frequent occurrence of medial hemorrhage in rat aortae, although more extensive, is in agreement with hemorrhages that have been described in or around areas of arteriosclerosis in man.[16] In man, medial hemorrhage about arteriosclerotic plaques probably originates from preexistent vasa vasorum, whereas in rats we believe that medial hemorrhage is secondary to a deterioration and rupture of the intima and media. Even though aortic rupture or aneurysm did not occur distant to the arch, focal medial edema and elastic fiber swelling or fragmentation were commonly observed in the abdominal aorta. Mural thrombosis has been invoked to explain the occurrence of arteriosclerotic plaques.¶ Hyaline mural

¶ References 17 and 18.

Fig. 12.—Adjacent section to that shown in Figure 11. The elastic fibers illustrate the width of the original media. Elastic connective tissue is not present in the areas of intimal and adventitial fibrosis. Weigert; × 130.

thrombi when observed in rat aortae, were secondary to extensive degeneration and destruction of the media and intima. Mural thrombi were searched for in the thoracic and abdominal aortae without success. Hypertrophy of the intima due to fibrous hyperplasia, which occurs in man,[19] was also seen in rat aortae. Intimal fibrosis or endothelial hyperplasia in the rat, however, occurred only in the arch of the aorta after medial injury. One of the most important observations in this study is that the intimal potential for fibrous and endothelial hyperplasia is tremendous following injury. Furthermore, the intimal hyperplasia is a manifestation of healing in response to injury. Our findings are in agreement with observations made after direct arterial trauma in animals # and the spontaneous appearance of similar lesions in man.[22] Neither lipophages nor cholesterol clefts were observed in these early lesions of intimal hyperplasia. This does not exclude the presence of minimal quantities of fat or lipids in such areas; however, it is reasonable to assume that, in the rat, medial degeneration and fibrous intimal plaques can develop in the absence of excessive collections of either fat or lipid. The frequency with which medial hemorrhage occurs in the Lathyrus-fed rat, however, does offer an opportunity to resolve whether intimal arteriosclerotic lipid plaques form in areas of old hemorrhage, as suggested by Winternitz and associates.[16]

SUMMARY

Water-alcohol-extracted sweet pea meal was fed to 17 controls and crude pea meal to 32 test rats. Neither bony deformities nor rupture of the aorta was encountered in 17 control rats. All of the test rats developed moderate to severe skeletal deformities. Aortic aneurysm or rupture was observed in 20 of 28 test rats. In our opinion the arterial degeneration induced by diet probably occurs in the following manner. Medial edema and fragmentation of elastic fibers occur first. After weakening of the media, either aneurysmal dilatation or medial hemorrhage, or both, may develop. The fibrous connective tissue proliferation, which may occur in the intima, the media, or the adventitia, is an early manifestation of healing in response to injury. The toxic factor probably acts indirectly because the production of arterial degeneration is modified by the concentration of casein included in the diet.

References 20 and 21.

REFERENCES

1. Stockman, R.: Edinburgh M. J. **19**:297, 1917.

2. Young, T. C. M.: Indian J. M. Res. **15**:453, 1927.

3. Sugg, R. S.; Sims, B. T., and Baker, K. G.: Vet. Med. **39**:308, 1944.

4. Geiger, B. J.; Steenbock, H., and Parsons, H. T.: J. Nutrition **6**:427, 1933.

5. Lewis, H. B.; Fajans, R. S.; Esterer, M. B.; Shen, C. W., and Oliphant, M.: J. Nutrition **36**: 537, 1948.

6. Lee, J. G.: J. Nutrition **40**:587, 1950.

7. Robinson, J. J., and Bast, T. H.: Anat. Rec. **59**:283, 1934.

8. Dasler, W.: Proc. Soc. Exper. Biol. & Med. **85**:485, 1954.

9. Ponseti, I. V., and Baird, W. A.: Am. J. Path. **28**:1059, 1952.

10. Wilens, S. L., and Sproul, E. E.: Am. J. Path. **14**:177, 1938.

11. Saxton, J. A., Jr.: Biol. Symposia **11**:177, 1945.

12. Dupuy, H., and Lee, J. G.: J. Am. Pharm. A. (Scient. Ed.) **43**:61, 1954.

13. Schilling, E. D., and Strong, F. M.: J. Am. Chem. Soc. **76**:2848, 1954.

14. McKay, G. F.; Lalich, J. J.; Schilling, E. D., and Strong, F. M.: Arch. Biochem. **52**:313, 1954.

15. Maniglia, R., and Gregory, J. E.: A. M. A. Arch. Path. **54**:298, 1952.

16. Winternitz, M. C.; Thomas, R. M., and LeCompte, P. M.: Biology of Arteriosclerosis, Springfield, Ill., Charles C Thomas, Publisher, 1938.

17. Duguid, J. B.: J. Path. & Bact. **58**:207, 1946.

18. McLetchie, N. G. B.: Am. J. Path. **28**:413, 1952.

19. Prior, J. T., and Jones, D. B.: Am. J. Path. **28**:937, 1952.

20. Taylor, C. B.; Baldwin, D., and Hass, G. M.: Arch. Path. **49**:623, 1950.

21. Prior, J. T., and Hutter, R. V.: Am. J. Path. **30**:637, 1954.

22. Crawford, T., and Levene, C. I.: J. Path. & Bact. **66**:19, 1953.

LYMPH NODE STRUCTURE IN CONTROL AND IN TUMOR-BEARING CFW MICE

MAURICE M. BLACK, M.D.
and
FRANCIS D. SPEER, M.D., New York
With the Technical Assistance of Jean Watts, B.A.

THE AXILLARY lymph nodes from different cases of human breast cancer show marked variations in the prominence of primary follicles, the degree of sinus histiocytosis, and the cellular and degenerative changes in the pulp.[1] Variability in the microscopic appearance of the regional lymph nodes was also found among different cases of human gastric carcinoma.[2] However, the consecutive series of breast cancer cases differed from the gastric cancer series in regard to the relative incidence of sinus histiocytosis and follicular hyperplasia in their respective regional lymph nodes. In the former series the incidence of a marked degree of sinus histiocytosis was about 30%, whereas such findings were rarely observed in the lymph nodes in the gastric cancer series. In both groups, however, the finding of sinus histiocytosis was associated with good survivals in almost all cases. A similar correlation between sinus histiocytosis of the regional nodes and a good survival was also observed in cases of melanoma and thyroid carcinoma. These findings suggested that sinus histiocytic reactions in the regional lymph nodes of cancer patients represented a visualization of some type of host resistance to the tumor.

It therefore seemed pertinent to ask (*a*) whether or not such changes occurred in the lymph nodes in noncancer cases, (*b*) whether such changes are limited to the regional lymph nodes in cancer cases or are found in other or all nodes, and (*c*) whether malignant tumors of different types growing in similar regions induce such changes in their draining lymph nodes with equal regularity. Since it is almost impossible to obtain any appreciable number of lymph nodes from different sites from normal persons, it appeared that any attempt to answer the above questions would have to employ experimental animals. We therefore initiated an investigation of the microscopic structure of various lymph nodes in control and tumor-bearing mice.

MATERIALS AND METHODS

A total of 204 CFW mice were employed in this investigation in the following groups: 45 control females weighing 21.3 to 36.2 gm., 45 control males weighing 20.0 to 38.9 gm., and 66 females bearing spontaneous mammary tumors. The tumor mice weighed 22.5 to 45.7 gm. The tumors ranged from 0.1 to 6.5 gm., with a mean weight of 1.33 gm. In addition, 4 females and 44 males bearing subcutaneously implanted sarcoma 180 (S180) were also studied. The tumors were implanted in the region of the right axilla and allowed to grow for periods of 7 to 32 days before the mice were killed. The weights of these tumors ranged from 0.2 to 4.5 gm., with a mean weight of 1.56 gm.

The mice were killed by crushing the cervical spine, and the lymph nodes were removed from both axillae and both inguinal regions. The nodes were labeled as to their site of origin, and, in the case of the tumor-bearing mice, notation was made as to the node group in closest proximity to the tumor. This procedure allowed a comparison to be made of the structure of the regional nodes with that of the more distant nodes. The lymph nodes were fixed in 10% neutral formalin, and routine paraffin sections were prepared and stained with hematoxylin and eosin.

The lymph node structure was classified according to the degree of prominence of the follicles and sinus histiocytosis. The grading system employed was the same as that described previously in our studies of human lymph nodes, viz., from 0 to 4 +.*

From the Department of Pathology, New York Medical College, Flower and Fifth Avenue Hospitals.

This study was aided by grants from the Helen Andreadis Foundation and the Leukemia Research Foundation.

* References 1 and 2.

A zero reading for sinus histiocytosis indicates that no sinus histiocytosis was found in any of the lymph nodes in the group, either because no sinusoids were observed in the nodes or because the sinusoids, when present, were empty or filled with inflammatory cells and vacuolated histiocytes. A 4+ sinus histiocytosis indicates that one or more of the nodes from a particular area shows marked prominence of the sinusoids which are compactly filled with large histiocytes having a finely granular eosinophilic staining cytoplasm and a vesicular nucleus which usually contains a fairly prominent nucleolus.

A zero follicular reading indicates that none of the lymph nodes of a group show follicles. A 4+ follicular reading indicates that at least one of the nodes shows very prominent follicles with secondary centers. Intermediate values for follicular prominence and sinus histiocytosis were designated in relation to the two extremes of the scale.

series the tumor ages varied from 7 to 32 days. Ten of the tumors had been growing from 20 to 32 days and ranged in weight from 1.0 to 4.5 gm. (mean weight 2.6 gm.), except one tumor, which weighed only 0.3 gm. However, in none of the 48 mice in the entire S180 series was sinus histiocytosis greater than 2+ found in the lymph nodes examined.

In the spontaneous breast tumor series the exact age of the tumors was not known, since the tumors were present at the time of purchase of the animals from Carworth Farms. However, since the size of the tumors may be roughly correlated with their age, it is possible to draw certain pertinent

TABLE 1.—*Per Cent Incidence of Various Degrees of Sinus Histiocytosis and Follicular Hyperplasia of Lymph Nodes*

Group	No.	Sinus Histiocytosis *					Follicles *				
		0	1	2	3	4	0	1	2	3	4
Control											
Male	45	73	22	2	..	2	29	16	47	9	..
Female	45	80	20	..	..	..	24	27	33	16	..
Breast cancer	66	27	33	23	14	3	23	24	30	20	3
S180											
Male	44	89	11	..	..	..	30	34	30	2	5
Female	4	50	50	..	..	..	50	..	50	..	..

* Based on highest reading found in any node.

RESULTS

In Table 1 we have indicated the per cent incidence of the various degrees of sinus histiocytosis and follicular hyperplasia found in the various groups of mice. In this listing we have employed the highest reading observed in the various nodes removed from the individual animal. In the control mice and in the mice bearing S180, the occurrence of sinus histiocytosis equal to or greater than 2+ was a rarity, observed in only 2 mice of the 138 in these categories.

In the females bearing spontaneous breast tumors, this degree of sinus histiocytosis in lymph nodes was found in 40% of the 66 animals studied.

It is conceivable that the age and size of the tumors might be a factor in the appearance of sinus histiocytic reactions of the lymph nodes. In this regard the following data should be considered. In the S180

conclusions from the data available. Of 29 mice bearing breast tumors weighing 0.1 to 1.0 gm. (mean weight 0.57 gm.), sinus histiocytic reactions greater than 2+ were found in 12 (41%). In those mice bearing tumors weighing 1.1 to 4.4 gm. (mean weight 1.95 gm.), the incidence of sinus histiocytosis greater than 2+ was 9/24, or 38%. Thus, there does not appear to be any correlation between tumor size per se and incidence of sinus histiocytosis. Since sinus histiocytic reactions of the lymph nodes were not found in mice bearing S180 within the age groups of 7 to 32 days and tumor weights of 0.2 to 4.5 gm., whereas mice of the same strain bearing spontaneous mammary tumors showed sinus histiocytic reactions even when the tumor size (and presumable age) was minimal, the lymph node structure seemed to be more closely related to the tumor type than to tumor age or size.

In contrast to this marked difference in the incidence of sinus histiocytosis, the incidence of follicular hyperplasia did not show any such distinction between the spontaneous tumor group and the various controls.

In Table 2 we have indicated the incidence of sinus histiocytosis readings equal to or greater than 2+, as observed in the various node groups examined. These data indicate that the axillary nodes are much more prone to show such structural changes than are the inguinal nodes. It will also be noted that this degree of sinus histiocytosis was observed most frequently in the axillary nodes on the same side as the tumor, although the difference between the tumor side and the contralateral side was small. Furthermore, a comparison of the degrees of sinus histiocytosis of the axillary nodes from the side of the breast tumor with those of the contralateral side, in individual cases, revealed the following facts: The lymph node reading of the tumor side was greater than that of the contralateral side in 34% of the cases, equal in 45%, and less than that of the contralateral side in 22%. Thus, in the majority of cases, the degree of sinus histiocytosis in the axillary lymph nodes of the contralateral side was at least equal to that found in the nodes on the same side as the tumor.

Additional information bearing on the incidence of sinus histiocytosis of the axillary lymph nodes in relation to the tumor site is presented in Table 3. It will be noted that sinus histiocytic reactions were found in the axillary lymph nodes in almost one-third of the cases in which the primary tumor was located in the inguinal region. Despite this, however, the inguinal nodes failed to show such reactive changes, regardless of the immediate proximity of the primary tumor.

TABLE 2.—*Per Cent Incidence of Sinus Histiocytosis Greater Than Two Plus*

Group	Axillary		Inguinal	
	Right	Left	Right	Left
Control				
Male	4	2	0	0
Female	0	0	0	0
Breast cancer *	28	21	0	0
S180 *				
Male	0	0	0	0
Female	0	0	0	0

* In the tumor-bearing mice, the tumor side is designated as the right side; the contralateral side is indicated as the left.

TABLE 3.—*Incidence of Sinus Histiocytosis of Axillary Lymph Nodes in Relation to Tumor Site*

Site	Cases, No.	Sinus Histiocytosis		Tumor Wt., Gm.	
		No.	%	Range	Mean
Axilla	17	9	53	0.2-4.4	1.2
Flank	5	1	20	1.2	1.2
Inguinal region	26	8	31	0.4-6.5	2.0
Multiple	10	6	60	0.5-1.9	1.1

In 16 of the mice bearing spontaneous breast carcinomas, metastases were found in the lungs. It is interesting to note that in none of these cases did any of the lymph nodes show sinus histiocytosis equal to or greater than 3+. Only 4 of the 16 cases (25%) had sinus histiocytosis equal to or greater than 2+. In the remaining 50 cases in which no metastases were found, the comparative values were as follows: sinus histiocytosis equal to or greater than 2+, 44%; equal to or greater than 3+, 22%.

It should also be pointed out that those mice having the higher degrees of sinus histiocytic reaction of their lymph nodes did not have proportionately larger and more necrotic tumors. If anything, the reverse was more frequently the case.

COMMENT

It is evident from the above data that sinus histiocytosis equal to or greater than 2+ was rarely observed in the axillary or inguinal lymph nodes from control male or female CFW mice. In contrast, the lymph nodes of approximately 40% of the mice bearing spontaneous mammary tumors showed sinus histiocytosis equal to or greater than 2+. Since these mice were of the same strain and of similar age and weight, the occurrence of an appreciable incidence of sinus histiocytosis in the tumor group and its virtual absence in the control groups is of particular significance and strongly suggests a cause-and-effect relationship.

That sinus histiocytosis is not simply a nonspecific reaction to a growing tumor with

its attendant tissue and tumor necrosis is indicated by the observations on the lymph nodes from the mice bearing implanted S180. The lymph nodes from this group failed to show any appreciable incidence of sinus histiocytosis of their axillary or inguinal nodes. These data provide further indication of the differences between the biological phenomena involved in transplantable and spontaneous tumors.[8]

It should be emphasized that, despite the fact that the lymph nodes showing sinus histiocytosis came almost exclusively from mice bearing spontaneous tumors, the corollary is not true, since less than half of the mice bearing the breast tumors had sinus histiocytosis of an appreciable degree. This variability in lymph node reactivity in the presence of apparently identical tumors is in conformity with our observations in human cancer cases.†

The present study also indicates that lymph nodes from different sites in the same animal may react differently to similar stimuli. Thus, the inguinal lymph nodes were much less prone to show sinus histiocytosis than were the axillary nodes, despite the fact that many of the tumors were in close proximity to the inguinal region.

Since the axillary lymph nodes on the side of the tumor tended to show a somewhat higher degree of sinus histiocytosis than did the contralateral lymph nodes, it might be inferred that sinus histiocytosis represents a purely local reaction of the draining lymph nodes. However, the phenomenon seems more complex than this, since the contralateral lymph nodes showed sinus histiocytosis equal to or greater than 2+ in 21% of the mice bearing the spontaneous breast tumors. Further, in individual cases the lymph node structure on the contralateral side was similar to that of the tumor side in the majority of the mice studied. These data would indicate that nodes at some distance from a tumor may participate in the sinus histiocytic reaction. We have also observed similar examples of sinus histiocytosis of distant nodes in individual human cancer cases. On the basis of the present study and our previous human studies, it would seem that the occurrence of sinus histiocytic transformations is influenced by the following factors: (*a*) the primary stimulus (tumor), (*b*) the distance from the tumor, and (*c*) the intrinsic responsiveness of the node itself. In regard to the last point, however, it should be mentioned that we have observed sinus histiocytosis in lymph nodes from a wide variety of sites, viz., axillary, inguinal, mesenteric, and cervical. It would therefore seem that the occurrence of sinus histiocytic changes in lymph nodes reflects the interplay of factors of both local and systemic origin.

Reference should also be made to the finding that sinus histiocytosis was less prominent and less often found in the tumor mice with lung metastases than in those without such dissemination. This observation is in accord with our findings in cases of human breast carcinoma, wherein the cases with sinus histiocytosis of the axillary nodes had a lower incidence of metastatic dissemination and superior survivals. The converse was true; namely, those cases with metastatic dissemination had a lower incidence of sinus histiocytosis than those without such dissemination.

The failure to find any appreciable change in the follicular prominence of the nodes of the different groups would lend further weight to the idea that sinus histiocytosis of the axillary nodes in breast cancer cases represents a relatively specific type of response related to the presence of the tumor. Certainly, further work on the factors responsible for the appearance of this type of lymph node reaction appears indicated. It might be mentioned that thus far we have been unsuccessful in our attempts to induce this type of response in the lymph node of mice by the implantation of heterologous tumors.

While sinus histiocytic reactions of a marked degree are uncommonly observed except in the presence of a malignant tumor, we are not implying that this is an exclusive and necessarily unique cause-and-effect re-

† References 1 and 2.

lationship. Furthermore, the present data do not allow conclusions as to whether we are dealing with a primary relationship or whether there are more generalized systemic factors operable, such as adrenal alterations.

SUMMARY

An investigation was made of the degree of sinus histiocytosis and follicular hyperplasia of the axillary and inguinal lymph nodes from control and tumor-bearing mice. It was found that sinus histiocytosis of the axillary lymph nodes occurred in approximately 40% of the female mice bearing spontaneous breast carcinomas. Such findings were rarely observed in control males and females and in mice bearing implanted S180. The inguinal lymph nodes did not show such change, even in mice with breast carcinoma.

No significant difference was observed in the degree of follicular hyperplasia in the different groups of mice.

The findings are discussed in terms of the specificity of the reaction and the biological phenomena involved.

REFERENCES

1. Black, M. M.; Kerpe, S., and Speer, F. D.: Am. J. Path. **29**:505-522, 1953.

2. Black, M. M.; Opler, S. R., and Speer, F. D.: Surg., Gynec. & Obst. **98**:725-734, 1954.

3. Black, M. M., and Speer, F. D.: A. M. A. Arch. Path. **57**:152-162, 1954.

News and Comment

ANNOUNCEMENTS

Symposium on Antimetabolites.—A symposium on The Antimetabolites—Their Modes of Action and Therapeutic Implications will be held in the Biltmore Hotel, New York, on Tuesday, March 1, 1955, under the auspices of the National Vitamin Foundation, Dr. Robert S. Goodhart, Scientific Director. The following program is planned:

Morning Session: Chairman, Dr. Paul L. Day, University of Arkansas, Little Rock, Ark.

1. Anti-Vitamin-E Stress Factors as Related to Fatty Peroxides.
   E. L. Hove, Alabama Polytechnic Institute, Auburn, Ala.
2. Thiamine Antagonists.
   L. R. Cerecedo, Fordham University, New York.
3. Vitamin K Antagonists.
   K. P. Link, University of Wisconsin, Madison, Wis.
4. Folic Acid Antagonists.
   J. H. Burchenal, Memorial Center for Cancer and Allied Diseases, New York.

Afternoon Session: Chairman, Dr. R. W. Heinle, The Upjohn Company, Kalamazoo, Mich.

1. Purine and Pyrimidine Antagonists.
   G. H. Hitchings, The Wellcome Research Laboratories, Tuckahoe, N. Y.
2. A Naturally Occurring Antimetabolite of Methionine in the Causation of a Disease.
   D. W. Woolley, The Rockefeller Institute for Medical Research, New York.
3. Pantothenic Acid Antagonists.
   O. D. Bird, Parke, Davis & Company, Detroit.
4. Vitamin B_6 Antagonists.
   W. W. Unbreit, Merck Institute for Therapeutic Research, Rahway, N. J.
5. Riboflavin Antagonists.
   J. P. Lambooy, The University of Rochester, Rochester, N. Y.

DECLINE IN MORTALITY FROM SYPHILIS IN MINNESOTA

E. T. BELL, M.D., Minneapolis

THERE has been a progressive decline in the incidence of syphilitic infections since about 1939. In New York State (exclusive of New York City) de Mello and Vought [1] noted a decrease of the reported cases of early acquired syphilis from 38.6 per 100,000 population in 1936 to 2.8 in 1952. During this period the reported cases of late-acquired syphilis decreased from 196 to 41.5 and congenital syphilis from 22.0 to 2.1, per 100,000 population.

Cowan and Shaw [2] found a decrease in the reported cases of primary and secondary syphilis in Michigan from 79 in 1946 to 4 in 1925 per 100,000 population. Total syphilis declined from 321 in 1944 to 112 in 1952 per 100,000 population. In Mississippi, Gray and associates [3] found the incidence of syphilis in pregnant Negro women 21% in 1938 and 10.9% in 1948. The rate of fresh infections reported to the U. S. Public Health Service [4] declined 90% between 1947 and 1952. The total reported cases of syphilis in the Continental United States declined from 486,000 in 1941 to 169,000 in 1952 (Moore [5]).

As to the mortality from syphilis there is much less available information. Usilton and associates [6] found that the total reported deaths from syphilis in the Continental United States had decreased from 15.0 per 100,000 population in 1939 to 8.0 in 1948; white males, 15.5 to 8.6; Negro males, 74.7 to 38.2; white females, 5.2 to 2.9, and Negro females, 36.3 to 16.0, per 100,000 population.

The purpose of this paper is to report the deaths from syphilis as recorded in the autopsies at the University of Minnesota during the period from Jan. 1, 1931, to Dec. 31, 1953. The deaths occurred in the cities of Minneapolis and St. Paul; and, since an autopsy is performed whenever permission can be obtained, it is believed that the sample is largely unselected. During the four-year period 1949-1952, autopsies were performed on 33% of the males and 24% of the females who died in this area.

The deaths from syphilis were due (*a*) to syphilitic aortitis with aortic insufficiency and cardiac failure, (*b*) to rupture of a syphilitic aneurysm, or (*c*) to some form of neurosyphilis. Occasionally a subject with neurosyphilis died of a ruptured aneurysm or syphilitic aortic insufficiency. The cases tabulated are only those in which a syphilitic lesion was the cause of death or an important contributory cause. Subjects with serological but no anatomical evidence of syphilis are not included in the tables. These data therefore pertain only to the mortality from syphilis and not to the incidence or curability of this disease.

The deaths due to acquired syphilis in males are arranged with respect to age in Table 1. The maximum percentage of syphi-

Department of Pathology, University of Minnesota.

Aided by a grant from the U. S. Public Health Service.

TABLE 1.—*Deaths in Males Due to Syphilitic Aortitis and Neurosyphilis From 1930 Through 1953*

Age, Yr.	1931-1940			1941-1948			1949-1953		
	Autopsies, No.	Deaths from Syphilis		Autopsies, No.	Deaths from Syphilis		Autopsies, No.	Deaths from Syphilis	
		No.	%		No.	%		No.	%
20-30	736	1	0.14	407	0	0.00	297	1	0.33
30-40	1,168	21	1.80	679	3	0.44	413	1	0.24
40-50	2,132	56	2.63	1,237	12	0.97	745	1	0.13
50-60	2,538	76	3.00	2,454	35	1.43	1,701	9	0.53
60-70	2,493	44	1.76	2,560	25	0.98	2,281	18	0.79
70-80	2,001	10	0.50	2,104	12	0.57	1,729	0	0.00
80-90	584	1	0.17	924	1	0.11	718	1	0.14
30-80	10,332	207	2.00	9,034	87	0.96	6,869	29	0.42

TABLE 2.—*Deaths in Females Due to Syphilitic Aortitis and Neurosyphilis From 1930 Through 1953*

Age, Yr.	1931-1940 Autopsies, No.	1931-1940 Deaths from Syphilis No.	1931-1940 Deaths from Syphilis %	1941-1948 Autopsies, No.	1941-1948 Deaths from Syphilis No.	1941-1948 Deaths from Syphilis %	1949-1953 Autopsies, No.	1949-1953 Deaths from Syphilis No.	1949-1953 Deaths from Syphilis %
20-30	690	1	0.15	368	1	0.27	168	0	0.00
30-40	832	4	0.48	515	0	0.00	236	0	0.00
40-50	1,026	15	1.46	744	8	1.04	461	2	0.43
50-60	1,155	14	1.21	1,053	6	0.57	725	0	0.00
60-70	1,190	10	0.84	1,231	5	0.41	1,036	5	0.48
70-80	1,015	0	0.00	1,271	1	0.08	994	2	0.20
80-90	300	0	0.00	597	1	0.17	522	0	0.00
30-80	5,218	43	0.82	4,814	20	0.42	3,452	9	0.26

litic deaths occurred in the sixth decade, and nearly all the deaths occurred between the ages of 30 and 80 years. It will be noted that the percentage of deaths due to syphilis in males who died between the ages of 30 and 80 years declined from 2.00% in the 1931-1940 decade to 0.42% in the five-year period 1949-1953. The mortality from syphilis is now about one-fifth of the 1931-1940 rate.

TABLE 3.—*A Comparison of the Frequency of Neurosyphilis and Syphilitic Aortitis*

	Neurosyphilis, No. of Cases	Syphilitic Aortitis, No. of Cases
1931-1940	87	166
1941-1948	25	85
1949-1953	6	34
Total	118	285

The deaths due to acquired syphilis in females are shown in Table 2. The percentage of deaths from syphilis in females is less than one-half that in males. There has been a decline in females similar to that in males.

The incidence of neurosyphilis and syphilitic aortitis in the three periods is shown in Table 3. Neurosyphilis appears to have declined more than syphilitic aortitis; possibly it is more favorably influenced by antibiotics.

The deaths from congenital syphilis are shown in Table 4. It appears that congenital syphilis has disappeared in this community.

SUMMARY

During the period from Jan. 1, 1931, through Dec. 31, 1953, autopsies were performed in the cities of Minneapolis and St. Paul on 29,901 males and 16,129 females between the ages of 20 years and 90 years. There were 26,235 males and 13,484 females between the ages of 30 and 80 years. In this group the deaths from syphilis in males (Table 1) declined from 2.00% in the 1931-1940 period to 0.42% in the 1949-1953 period. In females in the corresponding periods (Table 2) deaths from syphilis declined from 0.82% to 0.26%.

No instance of congenital syphilis was found in 2,743 autopsies on children less than 10 years of age during the years 1949-1953.

REFERENCES

1. de Mello, L., and Vought, R. L.: Decline and Fall of Syphilis in New York State, Am. J. Syph. **37**:545-552, 1953.

2. Cowan, J. A., and Shaw, W. D.: Venereal Diseases: A vanishing Problem, J. Michigan M. Soc. **52**:845-848, 1953.

3. Gray, A. L.; Usilton, L. J., and Iskrant, A. P.: Measurement of Trend of Syphilis in Mississippi, J. Ven. Dis. Inform. **31**:225-230, 1950.

4. Syphilis Cases and Deaths Reported in Various Countries, Epidemiol. & Vital Statist. Rep. **4**:176-179, 1951.

5. Moore, J. E.: Changing Pattern of Syphilis, 1941-1953, Ann. Int. Med. **39**:644-649, 1953.

6. Usilton, L. J.; Remein, Q. R.; Thorner, R. M., and Donohue, J. F.: Syphilis Mortality During the Period of the Fifth Revision of the International Lists of Causes of Death, Am. J. Syph. **37**:403-412, 1953.

TABLE 4.—*Deaths from Congenital Syphilis in Children Less Than Ten Years of Age, Including Stillbirths*

1931-1940 No. of Autopsies	1931-1940 Deaths from Congenital Syphilis	1941-1948 No. of Autopsies	1941-1948 Deaths from Congenital Syphilis	1949-1953 No. of Autopsies	1949-1953 Deaths from Congenital Syphilis
5,129	28	3,524	7	2,743	0

Books

Sandoz Atlas of Haematology. Compiled by E. Undritz and translated into English by A. M. Woolman. Price, $7.00. Pp. 91 with 579 illustrations. Sandoz, Ltd., Basel, Switzerland, 1952.

This is the second edition of this valuable atlas compiled by Dr. E. Undritz of the Sandoz Pharmaceutical Co., Basel, Switzerland, and translated into English for the first time by Dr. A. M. Woolman.

The atlas is divided into three sections. Section One deals with the fundamental principles of hematology, including terminology, functions of blood corpuscles, technique of blood and bone marrow examination, and normal values. Some of the descriptive terms used, such as "panmyelophthisis," "proerythrocyte," etc., may sound strange to some American pathologists and hematologists, but on the whole the terminology is satisfactory. It is regrettable that a few of the "normal values" listed are open to serious criticism; i. e., adult hemoglobin content of 14.5 to 20.0 gm. per 100 cc., and adult female erythrocyte count of 4.6 to 5.6 million per cu. mm. The chapter on functions of the various hematopoietic cells is necessarily limited, but it is also somewhat naïve.

Section Two is devoted mainly to classification and description of the morphology of blood corpuscles. This part is admirably detailed and contains some useful tables.

Section Three contains 44 color plates comprising 256 illustrations, which depict normal and pathological elements found in the blood and hematopoietic organs. Almost all of the material is from human subjects. With rare exceptions, these plates come very close to fulfilling the intention of the authors to reproduce blood cells as they actually appear under the microscope, within the limits of modern color-printing techniques. Each plate is accompanied by an explanatory note. This illustrative section is very complete, but it is perhaps unfortunate that no attempt has been made to emphasize which of the illustrations represent the commoner blood dyscrasias.

This atlas, particularly the illustrative section, may well be recommended to students, general physicians, and also to those more intimately concerned with diseases of the blood.

Coronary Heart Disease in Young Adults. By Menard M. Gertler and Paul D. White. Price, $5.00. Pp. 218, with 25 figures. Harvard University Press, Cambridge, Mass., 1954.

This volume will be valuable to those pathologists who have a particular interest in arteriosclerosis, especially those who are concerned with its anthropometric and biochemical aspects. No gross or histopathological findings are included in the study. The authors come to the conclusions that the young endomorphic mesomorph male is most prone to severe coronary disease, that elevation of the serum cholesterol-lipid phosphorus ratio is better correlated with the presence of severe coronary disease in young adults than either the elevation of serum cholesterol or lipid phosphorus, and that there is a correlation between the oxidation-reduction potentials of saliva and coronary arteriosclerosis.

Synopsis of Pathology. W. A. D. Anderson, M.A., M.D. Third Edition. Price, $8.00. Pp. 788, with 334 illustrations and 13 color plates. C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1952.

Anything approaching a comprehensive general coverage of modern pathology cannot be achieved between the covers of one book. But a summary of important points is possible and such is the goal of this book. It suffers from an order of incompleteness no greater than a good many less concise and more cumbersome volumes.

As in previous editions, the conventional organization into chapters on general pathology followed by a treatment of organ systems is employed. Etiology and pathogenesis are considered, as well as gross and microscopic anatomy. Little clinical correlation and no historical material are given. The book is well indexed and the references have been brought up-to-date. It is clearly and compactly written and is liberally illustrated with well-chosen gross and microscopic photographs.

The present edition includes no basic changes in presentation but, rather, a revision of material with additions based on recent work and thought in many fields. Certain omissions from previous editions have been corrected. One the whole, inaccuracies are few.

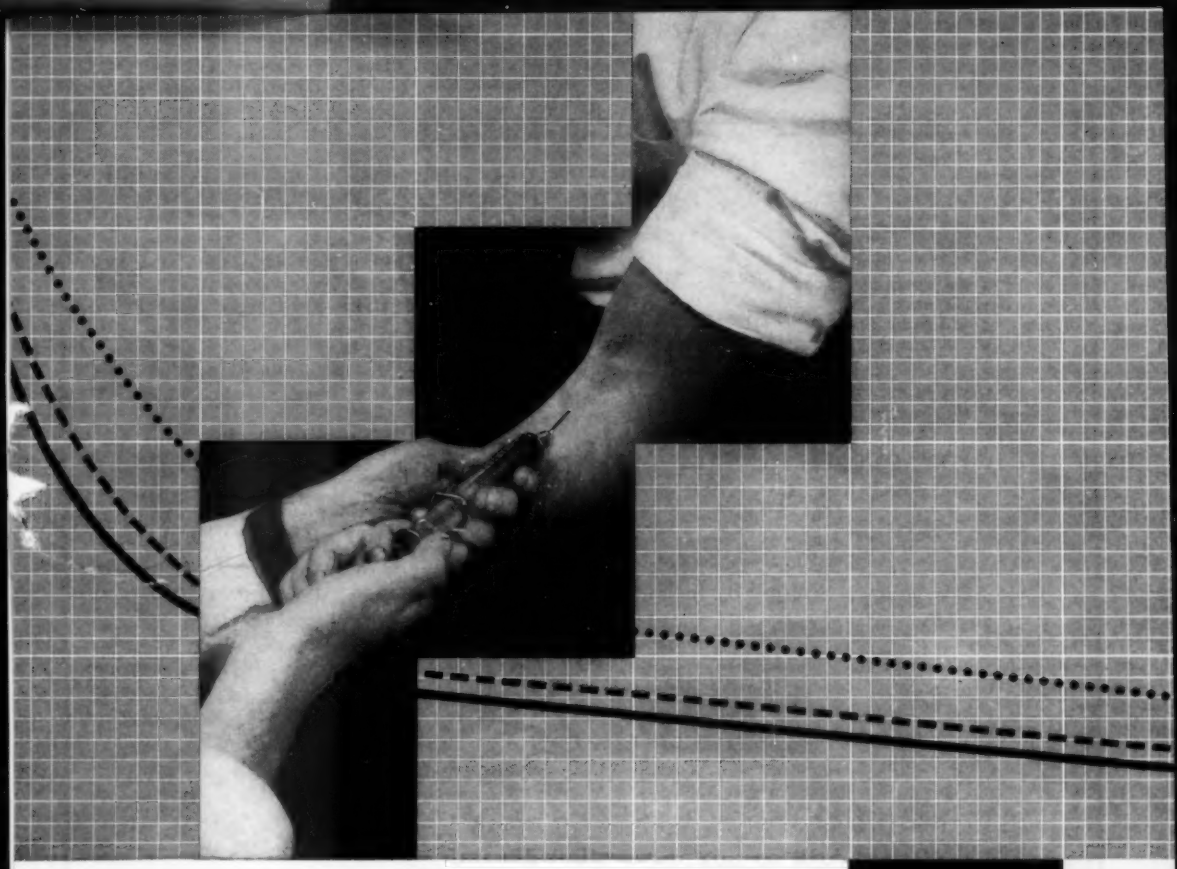
This synopsis perpetuates some deficiencies which are common to many pathology texts. A general consideration of humoral immunology should be included, with some treatment of immune mechanisms in inflammatory disease, in hypersensitivity, and in diseases of more obscure nature, such as glomerulonephritis and rheumatic fever. A discussion of chemotherapeutic modification of pathologic processes and also of pathologic changes actually produced by therapeutic agents would be useful because of the increasing proportion of disease rendered atypical or induced by treatment which is now encountered in the clinic and in autopsy material.

Legal Medicine. By R. B. H. Gradwohl. Price, $20. Pp. 1,093, with 222 illustrations. C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, Mo., 1954.

That forensic medicine is one of the most neglected specialties few physicians would deny. And most pathologists would readily agree that their knowledge of legal medicine is woefully inadequate. The author well realizes these deficiencies and the approach through this entire volume assumes neither a knowledge nor an understanding of legal terms and procedures. The entire design of the book is to enable the physician, and the pathologist in particular, to avoid costly and irreparable mistakes in the realm of legal medicine.

The coverage of this book is thorough. The author has asked well-recognized authorities in this country and Great Britain to contribute chapters on their particular fields. As a result, the chapters cover topics ranging from the history of legal medicine, the legal problems of autopsy examinations, and the legal aspects of the practice of medicine to various procedures for lie detection and the status of the medical expert witness. Extensive space is given to various forms of traumatic death and poisonings, to chemical and blood tests, and to psychiatric problems. Though the subject matter is markedly varied and does not lend itself readily to continuity, the volume has a uniformity of presentation that is a pleasure to read. Such uniformity reflects credit on the editing of the author.

It is unfortunate that the author is forced to recognize the serious deficiencies in the training of most physicians in legal medicine. Particularly is this serious in regard to the deficiencies in the training of pathologists, whose work often calls for an appreciation of forensic problems. Pathologists are interested in forensic medicine, as is well attested by the enthusiasm with which seminars on forensic medicine sponsored by the College of American Pathologists are received. Rather, the fault lies with the lack of training facilities, for only a few universities offer adequate instruction. The basic problem is that "Legal medicine is a specialty of general pathology. Hospital pathologists as a rule are not properly qualified in legal work. Until this fact is recognized, we shall continue to witness deplorable blunders. . . ." To avoid such blunders, every pathologist should have the present volume on his shelf, both for reading and reference. To overcome this problem in the future, all pathologists should be immediately concerned with the establishment of adequate training facilities in legal medicine.



blood will tell

When precise prothrombin time data are needed, Simplastin is a thromboplastin reagent of choice. Supplying the essential information swiftly and accurately, Simplastin performs a significant service in the laboratory "work-up" prior to anticoagulant therapy or surgery. For, "blood will tell"—more quickly and accurately—with Simplastin.

Bioassayed on normal human whole and dilute plasma, Simplastin assures sensitive, reproducible end-points. Preadjusted to optimum sodium chloride and calcium chloride content, it is available for immediate use merely with the addition of distilled water. Time-consuming and costly extra steps are eliminated.[1]

In the laboratory, Simplastin saves valuable time and effort... in therapy, it provides a dependable and accurate guide.[2,3]

Supplied in 2 sizes

Efficient
6-Determination Size for emergency use and for the smaller laboratory

Economical
20-Determination Size for the larger laboratory

Both sizes are supplied in boxes containing 10 vials. A trial supply will be sent promptly upon request. Available at leading laboratory supply houses; write for name of your local supplier.

1. Schilling, F. J.; De Natale, A., and Mottram, F. C.: Am. J. M. Sc. *222*:207 (Aug.) 1951.
2. Shapiro, S., and Weiner, M.: J. M. Soc. New Jersey *48*:1 (Jan.) 1951.
3. Shapiro, S., *et al.*: Am. Heart J. *40*:766 (Nov.) 1950.

Simplastin®

simple for the technician / *accurate* for the clinician

WARNER-CHILCOTT

what used to take *days*

To fully appreciate the significance of Autotechnicon's automatic processing, one needs only recall the tissue-processing practices in the pathologic laboratory prior to its introduction. Ten, twenty (or many more) containers of reagents spread along a table, over which a technician wearily presided, hour after hour, shifting tissues from one solution to another. The slow pace of the procedure was matched only by the uncertainty of the result.

By contrast, brisk overnight service is routine in today's Autotechnicon-equipped laboratory. Finished tissues are ready within short hours of surgery. Processing is completely mechanized . . . requires no human intervention or supervision. Results are predictably uniform; every step at every stage is strictly governed, timed to hairbreadth precision.

is now done in *hours* by the

Autotechnicon®

every phase of tissue processing *automatically* . . .
fixation, washing, dehydration, infiltration, staining

THE TECHNICON COMPANY, 215 East 149 Street, New York 51, N. Y.